Experiences with FOLIC ACID

TOM D. SPIES

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EXPERIENCES WITH
FOLIC ACID

PLATE I

Folic acid crystals.

EXPERIENCES WITH FOLIC ACID

by

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Dedicated to the following persons who have aided in the anemia studies during the past fifteen years:

Dr. W. B. BEAN
Dr. L. JOE BERRY
Dr. N. L. BROOKENS
Dr. A. B. CHINN
Dr. J. K. CLINE
Dr. ROBERT C. COGSWELL
Dr. H. S. COLLINS
Dr. C. A. DOAN
Dr. W. B. FROMMEYER, JR.
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Dr. R. W. VILTER
Dr. ROBERT M. WOOLFORD

PREFACE

The use of folic acid in the treatment of macrocytic anemia illustrates vividly the immense surge that can be given to a biologic field when it progresses to the point of having an important clinical application. The research on folic acid has been of intense interest to me because of its great scientific and practical value. It offered the prospect of a specific form of therapy with a pure chemical compound of known molecular structure. Of necessity, the study was done on human beings because there were no animal studies which had sufficient bearing on the subject to warrant the interpreting of results in terms of human disease. In the face of frank skepticism on the part of some of our professional colleagues we administered folic acid to persons with severe macrocytic anemia. After a few sleepless nights it was a great relief to see patients who had been critically ill sitting up in bed and clamoring for breakfast a few days after folic acid therapy was initiated. In justice to the conservative attitude of the physician toward the introduction of any new form of therapy for macrocytic anemia and the open skepticism toward any possible therapeutic value of folic acid, it is well to mention that all doubt has now given way to complete confidence — confidence that folic acid will improve the state of the blood and prolong the life of a person with pernicious anemia or the related anemias. The evidence at present is inadequate to permit a final statement whether or not folic acid is useful in either the treatment or the prevention of the

neural disturbances associated with pernicious anemia. I doubt that it will protect against such disturbances.

The rapidity with which scientific and therapeutic advances on folic acid have been made is unparalleled. In this new field there is already a vast amount of confusing and sometimes conflicting literature. It is to investigation under the scrutiny of enterprising physicians that much of the test material must now come for appraisal so far as its effect on human beings is concerned. Here the physician will find the points of approach discussed, and I stress particularly the paths of study to be pursued in arriving at the correct diagnosis. Many years of clinical experience in the fields of nutrition and hematology have led me to prescribe large doses of specific therapeutic substances for my patients. It is far better to prescribe too much than too little, too soon rather than too late. I have attempted to survey the whole field so that this discussion will prove serviceable not only to the physician who wishes to make practical application of what we now know of folic acid as an antianemic substance but also to the biochemist, the biologist and the student of nutrition. Much of this material is of immediate value in the practice of medicine.

The pathogenesis of pernicious anemia, sprue and other related macrocytic anemias is little understood. It is hoped and expected that this new and sharp tool will aid us in a better understanding. The relations between these anemias and the dietary deficiency diseases are much more intimate than was formerly supposed by many. A comparison of my clinical experience in the northern part of the United States, in the southern part of the United States, in Cuba and in Puerto Rico has been helpful in shedding light on the very complicated relationships of addisonian pernicious anemia, nutritional macrocytic anemia and tropical sprue.

It is impossible to mention by name all the persons who have aided in this study, but the work of many is described in the text.

December, 1946

—T. D. S.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

1. INTRODUCTION

A medical event of great importance occurred when, for the first time, a single synthetic chemical compound of known molecular structure was found to be effective in the treating of persons with pernicious anemia, nutritional macrocytic anemia and sprue. The recent discovery of folic acid was the result of work carried on in various laboratories and clinics and from widely differing approaches. It seems almost certain that these beginnings converged in some instances on the same substances and in others on substances that act similarly. In still other instances related group studies overlapped. At least four crystalline compounds have been obtained from liver, yeast and other sources. Their chemical structures are becoming known, and soon their relationships, one to the other, will be clarified. Now that these studies have, to some extent, become integrated, the results should be made available to the general physician. A review of the biologic aspects of the subject has been published by Berry and Spies,[1] but this new vitamin promises to be of such importance that it seems worth while to bring into one volume the various clinical investigations concerned with it. Those who have the interest and the time can dig deep into the biologic studies which eventually coalesced and resulted in the isolation and synthesis of folic acid. For the physician who has little time to study the voluminous literature on

TABLE 1.*—Observations Leading to Isolation and Synthesis of Folic Acid and Related Compounds

Name	Source	Biologic Activity	Chemical Nature
Norite eluate factor[2]†	Liver and yeast	Growth factor for L. casei and S. lactis	Basic; related to purines
Norite eluate factor[3]	Solubilized liver	do.	Acidic; not a nucleotide
Norite eluate factor[4]	Solubilized liver	do.	Purine and pyrimidine present
Folic acid[5]	Spinach	do.	Not a nucleotide
S. lactis R factor[6]	Unstated	Active for S. lactis; inactive for L. casei	
Crystalline L. casei factor[7]	Liver and yeast	Liver factor active for both organisms; yeast factor half active for S. lactis	Methyl esters of active principle
Folic acid[8–11]	Spinach	Same as folic acid above	Xanthopterin-like structure
Marmite[12]	Yeast	Cures dietary anemia in monkeys	
Vitamin M[13, 14]	Liver and yeast	Cures nutritional cytopenia in monkeys	
Xanthopterin[15–17]	Synthetic	Partially cures cytopenia in monkeys; cures trout anemia	
Vitamin B_c[18]	Liver	Cures chick anemia	
Factors R and S[19]	Yeast	Required in chick nutrition	
Crystalline vitamin B_c [20,21]	Liver	Active for chick, L. casei and S. lactis	Acidic; similar to flavins, aloxozines and pterins
Crystalline vitamin B_c [22]	Yeast digest	do.	do.
Crystalline vitamin B_c conjugate[23,24]	Yeast	Active for chick; 2–5% activity for L. casei and S. lactis	do.; molecule 2.8 larger
Crystalline L. casei factor[25]	Unspecified	Active for L. casei; inactive for S. lactis	Absorption spectrum unlike folic acid
Vitamins B_{10} and B_{11} [26,27]	Liver	Active for chick; inactive for bacteria	
Thymine[28]	Synthetic	Active for S. lactis	Pyrimidine
Crystalline L. casei factor[29]	Synthetic	Active for chick, rat, monkey, bacteria	
α pyracin and β pyracin [30–32]	Synthetic	Hemoglobin synthesis in chick	Lactone of pyridine

*Reprinted with slight modification from Berry and Spies.[1]
†References will be found on pages 102 ff.

the subject, Table 1 may be helpful, in showing how Lactobacillus casei, Streptococcus lactis R, the chick, the monkey, the rat, the pig and the guinea-pig have all been utilized in the basic studies.

Results of the clinical use of synthetic folic acid in human beings were first reported by Spies and his associates in September, 1945.[33] In November,[34] December[35] and January[36] they reported further studies which showed conclusively that folic acid given either by mouth or parenterally induced a striking hemopoietic

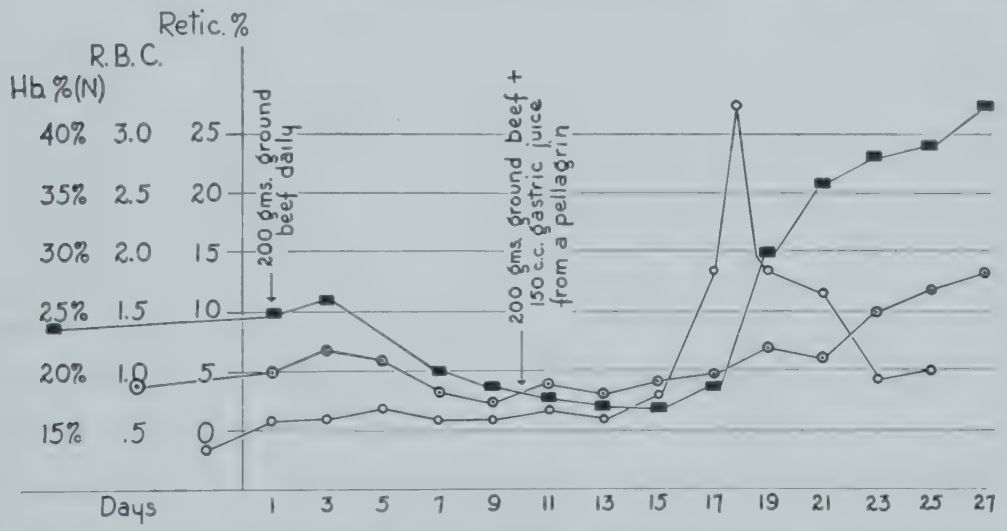


Fig. 1.—Hemopoietic response of patient with pernicious anemia to beef incubated with gastric juice from a pellagrin.

response similar to that which follows the administration of a potent liver extract. These results in cases of nutritional macrocytic anemia, addisonian pernicious anemia and sprue marked a milestone in the progress of our studies on anemia which have been under way for fifteen years. In 1930 I observed that many patients with severe pellagra had a macrocytic anemia which was cytologically indistinguishable from addisonian pernicious anemia. That this anemia was not due to the lack of the intrinsic factor of Castle was clearly indicated by Spies and Payne[37] in 1933 when they found that ground beef incubated with the gastric juice from pellagrins produced a remission in persons with addisonian pernicious anemia in relapse (Fig. 1).

In 1935 Spies and Chinn[38] reported that 63 per cent of their patients with severe cases of alcoholic pellagra had a macrocytic anemia. This observation convinced me that if we were ever to rehabilitate all the pellagrins who came under our care we would have to treat the associated macrocytic anemia. Accordingly, I began to search for chemical substances in liver and various foods which would cause a remission in anemia and began to build clinical centers where suitable patients could be attracted and treated.

Among the patients in the clinic in Birmingham* there is a high incidence of anemia, particularly macrocytic anemia. Although microcytic anemia occurs in many of our patients and has been studied extensively, it does not respond to folic acid and, accordingly, will not be discussed here.

The first clinical signs of anemia are so impressive that the diagnosis has been made in all civilized countries from early times. Because of the world-wide distribution of the anemias, physicians everywhere have sought the best method for their detection and therapy. The separation of the anemias into several forms was not possible until the development of modern technics for the study of blood. Nothing more impressive has been written concerning pernicious anemia than Addison's[39] following description:

"For a long period I had from time to time met with a very remarkable form of general anemia, occurring without any discoverable cause whatever; cases in which there had been no previous loss of blood, no exhausting diarrhea, no chlorosis, no purpura, no renal, splenic, miasmatic, glandular, strumous, or malignant disease. Accordingly, in speaking of this form of anemia in clinical lecture, I, perhaps with little propriety, applied to it the term 'idiopathic,' to distinguish it from cases in which there existed more or less evidence of some of the usual causes or concomitants of the anemic state.

*Developed chiefly by the aid of large grants from the Research Corporation of New York and from Eli Lilly and Company of Indianapolis.

"The disease presented in every instance the same general character, pursued a similar course, and, with scarcely a single exception, was followed, after a variable period, by the same fatal result. It occurs in both sexes, generally, but not exclusively, beyond the middle period of life, and so far as I at present know, chiefly in persons of a somewhat large and bulky frame, and with a strongly-marked tendency to the formation of fat. It makes its approach in so slow and insidious a manner, that the patient can hardly fix a date to his earliest feeling of that languor, which is shortly to become so extreme. The countenance gets pale, the whites of the eyes become pearly, the general frame flabby rather than wasted; the pulse perhaps large, but remarkably soft and compressible, and occasionally with a slight jerk, especially under the slightest excitement; there is an increasing indisposition to exertion, with an uncomfortable feeling of faintness or breathlessness on attempting it; the heart is readily made to palpitate; the whole surface of the body presents a blanched, smooth and waxy appearance; the lips, gums and tongue seem bloodless; the flabbiness of the solids increases; the appetite fails; extreme languor and faintness supervene, breathlessness and palpitations being produced by the most trifling exertion or emotion; some slight edema is probably perceived about the ankles; the debility becomes extreme, the patient can no longer rise from his bed, the mind occasionally wanders, he falls into a prostrate and half-torpid state, and at length expires; nevertheless to the very last, and after a sickness of perhaps several months' duration, the bulkiness of the general frame and the amount of obesity often present a most striking contrast to the failure and exhaustion observable in every other respect.

"With, perhaps, a single exception, the disease, in my own experience, resisted all remedial efforts, and sooner or later terminated fatally. On examining the bodies of such patients after death, I have failed to discover any organic lesion that could properly or reasonably be assigned as an adequate cause of such

serious consequences; nevertheless, from the disease having uniformly occurred in fat people, I was naturally led to entertain a suspicion that some form of fatty degeneration might have a share at least in its production; and I may observe, that in the case last examined, the heart had undergone such a change, and that a portion of the semilunar ganglion and solar plexus, on being subjected to microscopic examination, was pronounced by Mr. Quekett to have passed into a corresponding condition. Whether any, or all, of these morbid changes are essentially concerned, as I believe they are, in giving rise to this very remarkable disease, future observation will probably decide."

Addison did not mention involvement of the nervous system, and his classic description received little attention from the medical profession of his time. Biermer[40] reported in 1872 to the Medical Society at Zurich on 15 patients who had what he designated "progressive pernicious anemia." He described the patients in considerable detail and noted that they had alimentary tract disturbances. Pepper stressed the hyperactivity of the bone marrow.[41] Fenwick[42] stressed the important relationship of stomach atrophy to the development of anemia. Lichtheim[43] probably first directed attention to the neural disturbances of pernicious anemia. Thus, much was accomplished in learning the characteristics, symptoms and changes of the disease, but little in regard to therapy prior to the introduction of liver therapy.[44]

It is impossible in this introduction to make a comprehensive report on all the earlier work in the treatment of anemia in which substances were used that might now be found to contain "folic acid." Since liver therapy is so widely employed in medical practice, this aspect of the work will be briefly discussed. I shall attempt to give the background of thought and clinical experimentation which, combined with the evidence presented before, made the testing of L. casei factor in human beings seem an obvious step.

In 1931 Wills[45] demonstrated the curative effect of a yeast ex-

tract in macrocytic anemia of pregnancy which occurs commonly in India. Some years later Wills and Evans[46] found patients with tropical macrocytic anemia who did not respond to the more highly purified liver extracts which were of therapeutic value in pernicious anemia. This suggested the existence of an unknown hemopoietic factor in crude liver and autolyzed yeast extracts. This new factor could not be identified with thiamine, riboflavin or nicotinic acid but was present in a yeast-fullers' earth filtrate. Wintrobe[47] in 1939 reported maximal hemopoietic responses in five patients with pernicious anemia given dehydrated brewers' yeast at a level of 1–2 Gm. per Kg. of body weight per day. In one case liver extract given orally was more effective than the brewers' yeast. Nine patients on a maintenance level of 0.3–0.8 Gm. per Kg. of body weight per day remained well for four to 10 months. Reticulocytosis occurred in two patients given yeast extracts parenterally, but a significant increase in number of erythrocytes did not follow.

Several reports have appeared during the past few years concerning the anemia of pregnancy. Miller and Studdert[48] made a study of 23 patients, all of whom responded to specific therapy. Treatment could be discontinued in 14. Dietary deficiencies and vomiting were important factors associated with the etiology of the anemia. Free hydrochloric acid was present in the gastric juice of 18 patients. In these, marmite plus a good mixed diet was usually sufficient treatment. In the remaining patients, in whom no free acid was present or who did not respond to marmite, remission of the anemia followed injections of either a refined or a crude liver extract. A degree of iron deficiency often became apparent during therapy. Davidson, Davis and Innes[49] reported on 16 cases of anemia occurring during pregnancy which resembled addisonian pernicious anemia. A megaloblastic bone marrow was found. Perseverance and intensive therapy in refractory cases were considered to be of vital importance.

Anemia associated with deficiencies of the vitamin B complex

has been studied by Moore, Minnich, Vilter and Spies.[50] Among 50 patients with hypochromic anemia, 32 had a clinical vitamin deficiency. All were found to respond satisfactorily to ferrous salts administered orally. Brewers' yeast administered in some cases in doses of 25 Gm. three times daily did not alter the rate of increase of hemoglobin in comparison with that in patients receiving iron but no yeast. They[51] later reported that the oral or parenteral administration of a combination of niacin, thiamine, riboflavin, calcium pantothenate, pyridoxine, inositol, para-aminobenzoic acid and choline had no effect in correcting the macrocytic anemia in patients with pellagra or deficiency of the vitamin B complex. This observation was made on 25 patients with red blood cell counts under 3,000,000 whose diets had been deficient in animal protein and B vitamins for years. Clinical signs of vitamin deficiency such as glossitis, cheilosis and peripheral neuritis were usually found. Nine of 10 patients showed a reticulocyte response to daily injections of 4–8 U.S.P. antipernicious anemia units in the form of highly purified liver extracts, and eight of these showed a marked acceleration in rate of increase in erythrocytes. A reticulocyte response followed the feeding of beef muscle and an 80 per cent alcoholic extract of beef in other cases. These workers felt that the anemia was due to a prolonged dietary deficiency, often associated with poor absorption from the intestinal tract. Watson and Castle[52] reported the results of studies of three patients with nutritional anemia. All gave histories of dietary inadequacies, all had free hydrochloric acid, and two became anemic during pregnancy. The blood showed a macrocytic hyperchromic anemia with mild leukopenia and thrombocytopenia. Anisocytosis and poikilocytosis were less marked than in comparably severe pernicious anemia. The differential white cell count was normal. There were no neural manifestations. In two cases there was a prompt response to orally administered liver extracts immediately following therapeutic failure of liver extracts given parenterally, even in multiple U.S.P. units daily.

This showed a deficiency of some substance other than that effective in pernicious anemia. The third case indicated that intramuscular injections as a route for therapy were unsatisfactory because 10 times the normal amount was required.

In 1944 Sharp, Vonder Heide and Wolters[53] reported the results of preliminary clinical studies on the antianemic action of vitamin B_c in the form of a yeast concentrate. Ten patients, all of whom had been under observation for a year or more, were known to give no response to various types of antianemia therapy tested during this period. All had erythrocyte counts between 3,000,000 and 3,500,000 per cu. mm. and 9–10 Gm. of hemoglobin per 100 cc. of blood. The vitamin B_c concentrate was administered in amounts giving 600 gammas per day, and after the first week it was increased to 1,500 gammas per day. At the end of four weeks' treatment the hematocrit showed an increase, but otherwise there was little change in the blood picture.

Soon after the brilliant work of Minot and Murphy it became apparent that a potent extract from liver would be less difficult to administer than whole liver. A number of crude liver extracts were prepared and within two years Cohn had developed fraction G.[54] Fraction G became widely used for treating macrocytic anemias due to the absence or the deficiency of erythrocyte maturation factor (EMF). This factor was considered to be the end-product of a reaction between the extrinsic factor, found in food, and the intrinsic factor, found in the secretions of the gastric mucosa. On the basis of this concept, which was fostered by Castle, it was possible to classify the macrocytic anemias further on the basis of intrinsic factor or extrinsic factor deficiency. Thus, Castle considers that the macrocytic anemia of pernicious anemia is characterized by a deficiency of intrinsic factor, whereas the macrocytic anemias of pregnancy, of nutritional deficiency and of sprue are characterized by a deficiency of extrinsic factor.

With this concept in mind, many investigators directed their efforts toward study of the intrinsic factor, the extrinsic factor

and EMF. Some of the fractions obtained from liver were hematologically active, but the great majority of them produced no hemopoietic response whatever. Many other substances, such as brewers' yeast, hog stomach, kidney and brain, were tried with success. Several of these substances for many years had been known to have hemopoietic properties, but none of them came up to the hemopoietic standards of concentrated liver extract. Because of the efficacy of concentrated liver extract in inducing remissions in the EMF deficiency anemias, it became the standard of those students who were constantly assaying new substances and new liver fractions. It soon was apparent that the various preparations themselves varied considerably, depending on the source of extraction, the method involved and many other uncontrollable factors. It became obvious to some that liver might contain many substances that might be hemopoietically active. I learned that, early in the process of making liver extract, most of the folic acid present in mammalian liver was discarded. I then tested a number of potent liver extracts and found that some of them were almost devoid of folic acid. It occurred to me that the folic acid which was being discarded in the process of concentrating the antianemic factor of liver might itself have some antianemic effect. I realized that liver extract was a mixture of many chemical substances and, from past experience, knew that we were facing a difficult and tedious task. For a number of years we studied fractions of reticulogen, a commercial preparation of liver extract, prepared by Dr. J. K. Cline, and we tested the hemopoietic properties of many other nutrients and synthetic substances. Some of these had slight activity, but most of them had none. We were also working with concentrates; one of these was folic acid concentrate, furnished by Dr. E. A. Sharp. We had long known that a number of concentrated liver extracts, which were of high potency in treating pernicious anemia, contained only infinitesimal amounts of folic acid. Nevertheless the working hypothesis was that a number of chemical molecules could probably effect hemo-

poiesis and that folic acid might well be one of these even though it were not the active one in concentrated liver extract. With these facts and others in mind regarding the course of the disease, I decided to select suitable patients and give them folic acid under controlled conditions.

2. SELECTION OF SUBJECTS AND METHODS OF STUDY

Many of the patients included in the study have been under observation in the Nutrition Clinic of Hillman Hospital, Birmingham, for years. None of them have been studied for less than three months. Every patient admitted to our service in the hospital is referred from the Nutrition Clinic. They are referred originally by a physican or come directly as a result of knowing some patient who had been treated by us. Many of the patients who come directly are sent by friends with symptoms which they considered similar to symptoms which had been relieved following treatment in the Nutrition Clinic. When we have relieved a patient with pellagrous psychosis, many persons with mental diseases of a non-pellagrous nature are brought to the Clinic. Likewise, patients with pain in extremities of non-nutritional origin seek aid because they have heard of or known someone whom we relieved of painful and debilitating nutritional neuritis. Excruciating eye pain, arising from ulceration, relieved by riboflavin in one will bring many persons with ocular disturbances to us. Unless we can make a satisfactory diagnosis of nutritive failure in such persons, they are referred elsewhere for treatment and are not admitted in the Nutrition Clinic.

It is obvious that an accurate diagnosis is the very essence of successful clinical investigation, and from the beginning my associates and I have made every effort to obtain all pertinent information. In each case we obtain a careful medical history and make a thorough examination. This information is supplemented by that gained from dietary studies and indicated laboratory determinations.

The discussion of diagnosis in this report is limited to those aspects which led us to successful folic acid therapy. One very important laboratory procedure is a thorough blood examination. There are too many patients in the Clinic to do routine blood studies on every patient. Such studies are done, however, on all the patients who have clinical evidence of anemia and on those who have severe deficiency disease. The actual blood determinations are discussed later. Among the patients arbitrarily selected, there is a high incidence of microcytic hypochromic anemia and macrocytic anemia. Both types have been studied extensively. This report is concerned, however, with the effect of folic acid on persons with macrocytic anemia.

Although macrocytic anemias occur throughout the world, certain types are found much more frequently in one area than in another. In the Temperate Zones only sporadic cases of so-called nontropical sprue are seen, whereas in some tropical areas sprue is endemic. Addisonian pernicious anemia is infinitely more common in the Temperate Zones than it is in the tropics. Nutritional macrocytic anemia rarely occurs in the North Temperate Zone, but it does occur rather frequently in the South Temperate Zone and probably in the Tropics.

There is much difference of opinion among physicians regarding the pathogenesis of all these conditions, and the number of cases seen by most physicians is small. To study the disease as thoroughly as possible within a limited period of time, it seemed desirable to continue our studies in the centers in Cincinnati and in Birmingham and to collaborate with the University of Havana

Medical School, Havana, Cuba, and with the School of Tropical Medicine, San Juan, Puerto Rico, in the study of tropical sprue. Through the assistance of the University of Havana and the Institute of Nutrition of Cuba, a special ward was obtained at the Calixto Garcia Hospital in Havana, where Dr. Walter B. Frommeyer, Jr., and I, of the University of Cincinnati, worked in the closest of professional collaboration with Dr. Guillermo Garcia Lopez, Dr. Fernando Milanes, Dr. Jose Aristides Menendez and Dr. Ruben Lopez Toca, of the University of Havana. The University of Cincinnati and the School of Tropical Medicine at San Juan, Puerto Rico, began in November, 1945, a co-operative study of the treatment of tropical sprue with folic acid. Engaged with me in this study were Dr. Ramon M. Suarez, Dr. Ramon M. Suarez, Jr., and Dr. F. Hernandez-Morales.

In each of these three centers we used the following criteria for selecting patients before admitting them to any of our wards for the therapeutic assay of folic acid:

1. Macrocytic hyperchromic anemia.
2. Red blood count of 2,500,000 or less.
3. Color index of 1.0 or more.
4. Megaloblastic arrest of sternal bone marrow.
5. Flat oral glucose tolerance test.
6. Free hydrochloric acid in gastric secretions after histamine stimulation.
7. "Fatty stools."
8. Weight loss.

Base-line determinations were initiated immediately on the arrival of the patient in the ward. They were designed to be thorough and to insure the accuracy of the admission diagnosis. The base-line determinations are briefly outlined as follows:

Day of Admission

Weight on admission
Anemia test diet
R.B.C., W.B.C., Hgb. and reticulocytes of each day

1st Hospital Day

Sternal bone marrow aspiration
Packed cell volume
Kahn test
Icteric index
Nonprotein nitrogen
Blood vitamin levels (especially A, C, E, carotene)
Stool for ova, parasites and occult blood
Urine for routine analysis and urobilinogen test
History and physical examination

2d Hospital Day

G.-I. series and small intestine study

3d Hospital Day

Gastric analysis
Alcohol meal — 250 cc. 4% alcohol after fasting specimen
Histamine 0.69 mg. subcutaneously after fasting specimen
Titration with Toepfer's solution
pH titration
Rennin — Michaelis' method
Pepsinogen—Mett's method
Bile — nitric acid

4th Hospital Day

Glucose tolerance test (oral)

1. Glucose, 80–100 Gm., after fasting specimen
2. Specimen 30 minutes from time patient starts to take glucose
3. Specimen 1 hour
4. Specimen 2 hours
5. Specimen 3 hours

Occasionally the patient's condition made it impossible to complete all these observations in four days. In those rare instances we took whatever time was necessary, but therapy was not started until all base-line studies were finished.

On the patient's admission to the hospital, rigid dietary control was instituted. Every meal served to our patients was planned by Mrs. Dorcas Morgan or Miss Jean Grant, dietitians. Meat,

meat products, fish and poultry were excluded; only 1 pt. of milk, one egg and 3 level teaspoons of butter or other fat were allowed daily; all other foods were permitted in any amount desired. In a previous study of macrocytic anemia, 75 patients were restricted to this type of diet and none had a so-called spontaneous remission. We felt reasonably certain, therefore, that any hemopoietic response would be attributable to the folic acid rather than to any food the patients received. The food was prepared and was served by our own maids or orderlies under the supervision of a dietitian who checked and recorded the food intake of each patient after every meal. The details of the diet follow.

Diet for Anemia Patients

FOODS RESTRICTED

No meat of any kind
No meat soups or meat gravy
No fish
No chicken, chicken soup, chicken gravy
No turkey
No cheese
No raw vegetables
No salad dressing
No cream or ice cream
Only one egg a day, cooked very hard
Only *2 glasses (1 pt.) of milk a day*
Only *1 level teaspoon of butter* for each meal

FOODS ALLOWED

Bread
Crackers
Cereals
Rice, macaroni, spaghetti, noodles
Potatoes
Overcooked vegetables
Fruit, fruit juices
Cake
Pie
All other desserts or puddings except custard and ice cream
Jam, jelly, sugar
Tea, coffee

BREAKFAST

Fruit or fruit juices, as much as desired
Cereal, as much as desired
Toast or bread, as much as desired
Butter, 1 level teaspoon
Jam or jelly, as much as desired
Egg, one, cooked very hard
Milk, ½ cup (no more)
Sugar, as desired
Coffee or tea, if desired

DINNER

Potatoes, rice, macaroni, spaghetti or noodles, as much as desired
Overcooked vegetables, as much as desired
Bread, as much as desired
Butter, 1 level teaspoon
Fruit or dessert, as much as desired
Milk, *¾ glass (no more)*
Coffee or tea, if desired
Sugar, as desired

SUPPER

Potatoes, macaroni, spaghetti or noodles, as much as desired
Overcooked vegetables, as much as desired
Bread, as much as desired
Butter, 1 level teaspoon
Fruit or dessert, as much as desired
Coffee or tea, if desired
Sugar, as much as desired

BEFORE BED

Jelly sandwich or fruit

NOTE: The patient may have as much as he wishes of any of the foods allowed.

Blood examination.—Hematologic examinations were made daily.* They included white cell and erythrocyte counts, hemoglobin determinations and reticulocyte counts. Certified Trenner pipets were used for both the white cell and the erythrocyte counts. The hemoglobin content of certain patients was determined in grams by means of a Leitz colorimeter. In other patients it was determined in grams by means of an Evelyn colorimeter. The reticulocytes were counted in wet preparations by the use of a modified brilliant cresyl blue solution of Dameshek. Permanent fixed preparations of blood smears were made of all patients on admission and just prior to treatment, and once or twice per week thereafter cell volumes were determined on oxalated venous blood by means of Wintrobe hematocrit tubes. In each case bone marrow was obtained by sternal aspiration prior to treatment. An effort was made to obtain another specimen at the peak of reticulocytosis, and still another specimen was obtained when the

*These were done with the assistance of Clemencia Benitez Gautier, Margaret H. Caldwell, Belle Culver, Jane Davis, Ann English, Doris Godwin, Georgia Gwinner, Betty H. Hadley, Madeline R. Hill, Mary B. Koch, Virginia Minnich and Mary Sax.

reticulocyte content returned to normal. Differential counts were made on preparations stained with both supravital and Wright-Giemsa stains. Icteric index was determined on those patients whose skin had a lemon-yellow color. A few serum iron determinations were made in some of the cases of tropical sprue from Cuba.*

In all the patients indicated blood chemistry determinations were made. In Havana we routinely tested the serum protein, blood calcium, phosphorus, potassium, amylase and lipase contents. In Birmingham we routinely determined vitamin A, E, C and carotene contents.

Alimentary tract studies.—In each of the centers a thorough study of alimentary tract function was made before, during and after therapy. The physical examinations included all accessible parts of the alimentary tract. In Cincinnati we have carefully studied a group of persons with addisonian pernicious anemia who have been maintained on folic acid for 10 months. In San Juan we have made extensive studies of a large series of persons with tropical sprue in order to determine whether or not they could be maintained in good health with folic acid therapy. Although repeated gastric analyses have been done on patients in each of the centers, in Birmingham particular emphasis has been placed on this aspect of the study. In Havana we have made and are making intensive chemical, bacterial and parasitic studies of the stools. Gastrointestinal studies are done on all patients but in Havana they are done routinely before, during and following therapy. These studies have been paralleled by studies of normal persons and of persons with tropical sprue who were not given therapy. In every case of tropical sprue, 24 hour stool specimens were collected in a large glass graduated container. The stools were examined each day, the appearance was noted and the volume measured. Each patient was questioned every morning by his physician in regard to all the details concerning

*Made by Virginia Minnich and Dr. Carl Moore.

his bowel movements, and his statement was recorded. In Cuba samples of the stools were taken for study; many fatty acid determinations were made by the method of Labbé and Larue.*

Twenty-five patients were selected for study of (1) the relationship of bacterial flora of the alimentary tract to folic acid therapy, (2) the relationship of intestinal parasitism to folic acid therapy and (3) the effect of folic acid on the alimentary tract as shown by roentgen changes before, during and after treatment with folic acid.† In these patients the large intestine was examined by curettage of the most altered portion of the mucosa of the rectum and sigmoid during rectosigmoidoscopy. The Miller-Abbott tube was utilized to study the small intestine, and, by the technic of Harris, 3–5 cc. of material was collected from the jejunum, the jejunoileal junction and the ileum. Each specimen thus obtained was examined microscopically while in the fresh, warm state. Immediately thereafter, a few drops of Lugol's solution were added to the specimen and microscopic examination was done by the flotation method of Willis. Bacteriologic culture was made on each specimen according to "standard bacteriologic technic." The individual specimens were planted on each of several different mediums in an effort to obtain as many positive cultures as possible.

The same patients in Havana were studied radiographically by means of frequent gastrointestinal series.‡ To avoid all possibilities of allergies to milk, barium sulfate was suspended in water and administered, and pictures were taken at 15, 30, 45 and 60 minute intervals. Follow-up pictures were taken at two, three, four, five, 12 and 24 hour intervals. The whole procedure was repeated two or three weeks after the initiation of folic acid therapy, again at the time the patient was ready to be discharged from the hospital, and once again two months after discharge.

*These determinations were made by Robert Johnson.
†In collaboration with Drs. Milanes, Curbelo, Rodriguez and Kouri.
‡These studies were made by Dr. Hernandez Beguerie and me.

The same procedures were conducted on normal subjects and on the control patients with sprue who did not receive any therapy but whose diet was the same as that of the patients.

With the aid of many professional colleagues and nurses,§ these hospital studies in the various centers were made as thorough as possible. The nurses were invaluable in the study of folic acid therapy. One or more supervised the care of each patient. All of the folic acid was weighed on an analytical balance and was administered by one of them in the amount prescribed and at the time ordered. They supervised the collection of all specimens and aided in the making of gastric analyses and glucose tolerance tests. They carried out these special duties in addition to giving the patients the best of general nursing care.

History.—In the centers at the Cincinnati General Hospital and Hillman Hospital, Birmingham, comprehensive medical and dietary histories were obtained and a careful physical examination was made. Figures 2, 3 and 4 show forms which illustrate in general the type of data recorded. Although no form is entirely satisfactory in every detail in each case and although we frequently modify these forms in the individual case, those presented here may be of some help to the physician.

As has been pointed out, in the two centers in the Temperate Zone — Cincinnati and Birmingham — most of the patients in the anemia clinics selected for study have either pernicious anemia or nutritional macrocytic anemia, while in Havana and San Juan — the two centers in the Tropical Zone — most of the patients studied have tropical sprue. Accordingly, the information obtained and the studies made differed somewhat. In the study of sprue in San Juan, with Dr. Ramon M. Suarez, Sr., Dr. Ramon M. Suarez, Jr., and Dr. F. Hernandez-Morales, the procedure outline on page 37 was routinely carried out, and a similar routine

§The nurses were Betty Duncan, Mariana de la Fuente Garcia, Maria del Carmen Gonzalez Vila, Virginia Hawkins, Jane Mann, Alice Rogers, Monette Springer and Flora Villate.

NAME_____Clinic Hospital Examiner Date

Sex Color Age Weight

Family History of Pellagra (X = had disease; D = died of disease; - = negative)

Mother Brother Uncle Husband
Father Sister Aunt Wife Children

Past History: First attack No attacks
Predisposing Precipitating Age of children Length of
Operations 1 6 Lactation
Infections 2 7
No. pregnancies 3 8
Menstrual 4 9
5 10

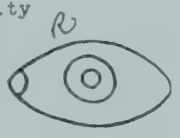
Chief complaint Date of onset

Present Illness:
Tinnitus Headache
Miscellaneous Dizziness

Eyes: Burning Blurred vision
Discharge Lacrimation
Night blindness Photophobia

Ears: Hearing
Nose:
Mouth: Teeth: Upper-in out Lower-in out Tongue-sore red Onset Lips-sore red onset
Sore throat Salivation
Skin: Dermatitis Location Onset

Mental Symptoms: Onset
Hallucinations
Insomnia
Nervousness
C.R. Dyspnea P.N.D. Palpitation Cough
Orthopnea Angina Depend. edema Hemoptysis
G.I. Anorexia Neurological: Pain in legs
Constipation arms
Vomiting Parasthesia
Diarrhea weakness of
Pain in stomach
Proctitis General: Weakness
Easy fatigability
G.U. Vaginitis Skin - burning
Urethritis itching
Perineal lesions Weight loss



Physical Examination:
Developed - well Nourished - well
moderately moderately
poorly poorly

Eyes: Pupils React to L. & A.
Conjunctiva
Sclera Circumcorneal injection Inflamed
Cornea Pterygium
Opthalmoscopic Arcus Nystagmus
Slit lamp

Hair color:

Nose: Sharkskin Nares Nasomalar Nasolabial

Fig. 2.—Form for recording data obtained from physical examination.

Teeth: Caries Pyorrhea

Mucous membranes - buccal Lips (mottling (fissures (atrophy Angles - Cheilosis crusts fissures scars atrophy pallor redness maceration

Tongue: Color
edema
atrophy
tooth markings
coated
ulcers
fissures
papillae - slit lamp
atrophic
hypertrophic
cobblestone
degree

Palate - soft
hard

Neck: Trachea Red center - number
Thyroid

Nodes:

Skin - mottling sweating purpura Triple response Spider angiomata

Face	Knees	Shoulders	
Neck	Shins	Elbows	
Chest	Feet	Forearms	
Back	Toes	Hands	
Abdomen	Perineum	Fingers	Nails

Chest: Heart Lungs B.P.

Abdomen:

Extremities:

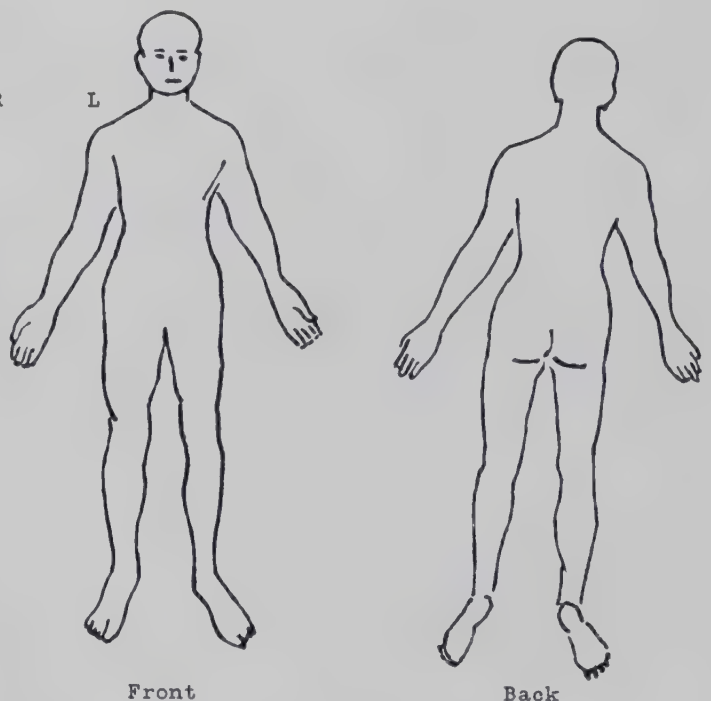
Miscellaneous:

Neurological: R L
Pain on soles
Pain on calf
Knee jerk
Ankle jerk
Babinski
Parasthesia: arm
leg
Vibration: arm
leg
Dynamometer

Remarks:

Deficiency:
Nicotinic acid
Thiamin
Riboflavin
Vitamin B_6
Iron
Vitamin A
Vitamin C
Extrinsic Factor

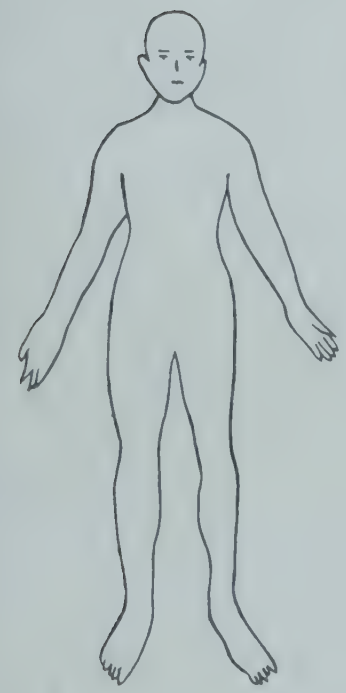
\+ mild
++ moderate
+++ severe



Front Back

Fig. 2.—*Continued*

NEUROLOGIC CHART



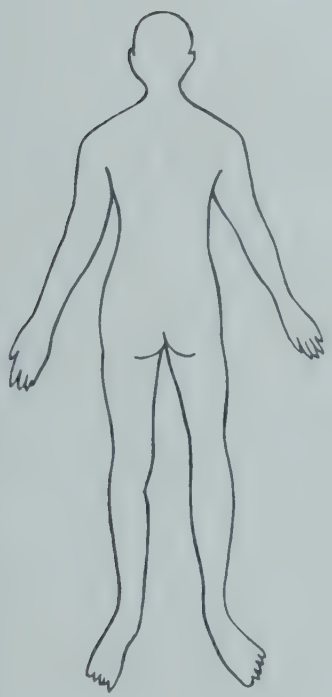


Fig. 3.—Form for recording data obtained from neurologic examination.

NUTRITION HISTORY

Name— *Age*— *M* *F* *Date*
Address— *Race*—

FOODS USED—

Dairy Products and Fats—
- Whole milk —
- Buttermilk—
- Skimmed milk—
- Canned milk—
- Cheese—
- Butter—
- Other fats—
- Eggs—
- Mayonnaise

Meats
- Lean meat—
  - Beef—
  - Lamb—
  - Pork—
  - Veal—
  - Liver—
- Fat meat—
- Fish—
- Fowl—

Vegetables and Fruits
- Potatoes, Irish—
- Potatoes, Sweet—
- Dried vegetables—
- Greens—
- Carrots—
- Tomatoes—
- Other vegetables—
- Orange—
- Grapefruit—
- Dried fruits—
- Other fruits—

Breads
- Cornbread—
- Biscuit— (Enriched Flour or Plain)
- Loaf bread—(Whole Wheat, Enriched,
- Crackers— Plain White)

Cereals
- Cornflakes—
- Oatmeal—
- Grits—
- Rice—
- Others—

Beverages
- Coffee—
- Tea—
- Soft drinks—

Desserts, Sugars, Miscellaneous
- Desserts—
- Sugar—
- Syrup—
- Honey—
- Jam, jelly—
- Candy—
- Peanut butter—

Vitamin Preparations—
Appetite—
How long was this diet used—
Variations in kind and amount of food used—
Foods Liked—
Foods Disliked—

Economic Status
- Number in family: Adults—
  Children— Boys—
  Girls—
  Ages—
- Urban—
- Rural— Total acres of land—
  Acres cultivated—
- Rent— Share— Own—
- Income (source and amount) —

Food Supply
- Food produced at home—
- Garden: summer— winter—
- Vegetables grown—
- Foods canned:
  - Fruits—
  - Vegetables—
  - Meats—
- Cows— Hogs— Chickens—

(What months are eggs and milk and meat available)

Deficiencies in Diet
- Protein—
- Calories—
- Calcium—
- Phosphorus—
- Iron—
- Vitamin A—
- Thiamine—
- Vitamin C—
- Riboflavin—
- Nicotinic Acid—

Alcohol, Tobacco
- Alcoholic Beverages—
- Snuff—
- Tobacco—

Fig. 4.—Form for recording nutrition history.

NAME ..... WARD ROOM .....

Order given		ORDER	Order discontinued	
Date	By whom		Date	By whom
		SPRUE ROUTINE*		
		1. *a*) Admission bone marrow aspiration		
		b) Bone marrow aspiration 3 days after initiation of treatment		
		c) Bone marrow aspiration q. month		
		2. Vibratory test		
		3. Blood chemistry (glucose, N.P.N., urea nitrogen, cholesterol, phosphorus, calcium, phosphatase, total proteins)		
		4. *a*) Plasma ascorbic acid (Dr. Munsell)		
		5. *a*) Admission glucose tolerance test, oral		
		b) Admission glucose tolerance test, intravenous (use 50 Gm. glucose)		
		c) Discharge glucose tolerance test, oral		
		d) Discharge glucose tolerance test, intravenous (use 50 Gm. glucose)		
		6. Hanger test		
		7. Takata-Ara test		
		8. Fragility and tourniquet tests		
		9. Heterophile antibody reaction (q. two wks., to het. antibody agglut.—5 cc. coagulated blood)		
		10. Bleeding and coagulation times		
		11. Venous pressure		
		12. Circulation time		
		13. Vital capacity		
		14. *a*) Gastric analysis		
		b) Discharge gastric analysis if achylia is present		
		15. Complete blood count and hematocrit studies (Mon. and Thurs.)		
		16. Reticulocyte count (daily)		
		17. Prothrombin time		
		18. Rectosigmoidoscopy (q. month)		
		19. Urobilinogen *a*) in urine *b*) in feces		
		20. Icteric index and van den Bergh reaction		
		21. Specify color, amount, consistency and character of stool (on nurses' note)		
		22. X-ray small intestine and barium enema		
		23. X-ray of heart and lungs		
		24. Blood volume — fasting		
		25. Electrocardiography		
		26. Gastroscopic examination		
		27. Dark adaptation test		
		28. Determination of fat and fatty acids in stool — biochemistry		
		29. B.M.R.		
		30. Weigh patient every 3 days		

*Developed at the School of Tropical Medicine, University Hospital, San Juan, P. R.

was followed in Havana. The various data were determined for each patient and recorded in his history. In the various study groups these outlines were completed and in addition the age, race, color, sex, marital status and occupation of the patient were placed in the written records.

PREVIOUS ADMISSIONS. Earlier admissions were summarized whether to our service or to any other hospital. In this summary were included the date of admission, date of discharge, diagnosis, pertinent physical findings, laboratory data, the treatment and the response. If the patient ever had anemia, all information about it was written in great detail, including the date of therapy and the type of response, the number of days of special therapy and the increase in the red blood cells, hemoglobin, white blood cells and platelets.

PRESENT ILLNESS. The patient's disease was recorded from the very first day that he stated he became ill. This record was made as complete as was humanly possible, including all positive or negative statements concerning any of his symptoms.

PAST HISTORY. All accidents, injuries, operations and illnesses were recorded. If the patient's history was essentially negative, it was so listed.

FAMILY HISTORY. The health and status of the mother, father, brothers and sisters of the patient were included. We sought for positive and negative statements concerning all known familial hereditary diseases and questioned the patient regarding the occurrence of nutritional deficiencies in any relative, including grandparents, aunts, uncles and cousins.

MARITAL HISTORY. This included the health and status of wife and children. Nearly always it was possible to interview each one of them and so obtain this information directly. We went into the habit history of each patient in great detail and also into his economic history in a most thorough manner.

DIETARY HISTORY.* We obtained, as far as possible, all infor-

*Taken by Miss Jean Grant or Mrs. Dorcas Morgan.

mation concerning the patient's past dietary history and always asked what he considered a typical day's dietary. This was done by listing breakfast, lunch and supper. Frequently we went to the patient's home to check on this information and were able sometimes to learn the exact amount he had at each meal. We were particularly interested in his intake of green vegetables and animal protein. Needless to say, much pertinent information could not have been obtained without a complete dietary history, often extending over a long period.

Physical examination.—Since we were consistently looking for any complications, a thorough system review was made. We asked all the questions we could think of pertaining to

Ears, eyes, nose and throat
Cardiorespiratory system
Gastrointestinal tract
Genitourinary tract
Neuromuscular system
Pulse, respiration, temperature and blood pressure

The record forms were used in the effort to obtain and list in detail all general information. The general statement in the physical examination is much more thorough than that ordinarily made because we were checking not only the past state of development, nourishment and declaration of illness but also the patient's general reaction to his disease concerning both his physical and mental make-up. We were concerned with whether he was weak or strong, whether he was alert and co-operative, or semi-comatose or unco-operative.

HAIR. The color, texture and amount of head hair were noted, and also the color, texture, amount and distribution of general body hair. Positive statements regarding these characteristics were always made.

SKIN. The skin was described in great detail. The description

included the color, i.e., whether it was icteric, whether it had pigmentation or whether it had erythema. Its texture was also recorded —is it thick, inelastic, loose or atrophic? Positive statements were made as to whether or not petechiae or telangiectases were present and whether or not acute or chronic pellagrous dermatitis was present.

LYMPH NODES. All lymph nodes were palpated, including the submental, ante- and postauricular, suboccipital, anterior and posterior cervical, axillary, epitrochlear and inguinal. If none of these nodes were palpable, the notation read "none palpable." If they were felt, we indicated their location and number and made a statement whether or not they were tender and whether they were discrete or matted.

HEAD. In the examination of the head its shape was noted. Of course, we listened for bruits and felt for exostoses and always observed the general appearance of the patient's head and face. For example, the typical patient with addisonian pernicious anemia has squat features and large ears.

EARS. The size of the concha and the condition of the canals and drums were recorded. The question of hearing was not considered at this point because it is included in the neurologic examination.

NOSE. The most important examination is that of the mucous membrane, which in acute pellagra is scarlet or violet and in pernicious anemia is more likely to be pale and icteric. If there were no abnormalities of the nose, it was so stated.

EYES. Both the palpebral and the bulbar conjunctivas were described. A positive or a negative statement was made concerning the vascularity of the conjunctivas. The shape of the pupils was recorded and their reaction to light and in accommodation. Extra-ocular muscle function was described and a fundus examination always made. Particular emphasis was placed on the degree of retinal arteriosclerosis.

MOUTH. The lips, angles, buccal mucous membrane, gums,

tongue and, finally, the teeth and pharynx were examined. All abnormalities were described.

NECK. Whether or not the thyroid was palpable was always indicated because this is sometimes important in determination of the etiology of nutritional diseases. This is the only positive statement included under Neck. If there were no abnormalities, the statement read "essentially negative."

CHEST. Under this heading were included observations of the bony thoracic cage; not included were observations of the lungs and heart.

LUNGS. A statement was made as to the findings on percussion and auscultation. When there were no abnormalities, we stated that the lungs were clear on P and A.

HEART. The size, rate, rhythm and presence or absence of murmurs were recorded. Notation was made whether or not gallop rhythm was found and whether or not A_2 was greater than P_2.

ABDOMEN. Its general appearance was observed. We noted whether or not the liver, kidney and spleen were palpable. If the liver, kidney and spleen were enlarged, we noted whether they were tender or nontender and included an estimation of the degree of enlargement in terms of centimeters. A positive statement of the presence or absence of abnormal masses or areas of tenderness was made. For males the status of the inguinal rings was included.

GENITALIA. Any abnormality of the penis and testicles was described. If there were no abnormalities of the genitalia, a statement to that effect was included.

RECTAL EXAMINATION. This was always made since many of our male patients are old and since carcinoma of the prostate frequently precipitates nutritional diseases. Any external abnormalities of the rectum were described. The size and consistency of the prostate were noted. If it was abnormal in size and consistency, the abnormalities were recorded in detail.

EXTREMITIES. The nail beds of the fingers and toes were ex-

amined and described and the status of the peripheral vessels was noted. The radial pulses on both sides were always palpated, as were the dorsalis pedis pulses. If these pulses were present and of normal volume, it was so stated. The presence or absence of pitting dependent edema was noted. No skin or neurologic findings were included.

CRANIAL NERVES. If the twelve cranial nerves were normal and intact, it was not deemed necessary to list them specifically. However, if any abnormality was found in any of the cranial nerves, the particular nerve received a complete neurologic examination.

MOTOR. Under this heading were included notations on the presence or absence of fibrillation, tremors, generalized or localized paralysis and on muscle strength and muscular atrophy. It is essential that the presence or absence of all signs be recorded.

SENSORY. The status of perception to light touch, pain (pinprick) and vibration, position sense and nerve, trunk, calf and other muscle tenderness were noted here.

REFLEXES. The following reflexes were included in every neurologic examination:

Reflexes	Right	Left
Biceps		
Triceps		
Radial		
Hoffmann		
Abdominals, upper and lower		
Cremasteric		
Knee Jerks		
Ankle Jerks		
Plantar (The plantar reflexes include the Babinski, Gordon, Oppenheim and Chaddock. If these were all negative, plantar reflexes under that heading were listed as negative.)		

A positive or negative statement regarding the presence or absence of pathologic reflexes was always made.

Following the physical examination were listed pertinent laboratory data, including blood counts, gastric analysis, bone marrow studies, glucose tolerance and other tests used to confirm the diagnosis. Following the laboratory data, we gave our impression.

Impression.—In the order of prominence and severity each of the physiologic and anatomic diagnoses was listed. The impression was stated as completely as possible.

Diagnosis.—As soon as the foregoing information had been acquired for each patient, every effort was directed to the proving of the diagnoses. Because the pathogenesis of none of the macrocytic anemias is thoroughly understood, there has been much confusion concerning their diagnosis. Our studies have shown that addisonian pernicious anemia and nutritional macrocytic anemia are cytologically indistinguishable and that the best single differentiating feature is the presence of free hydrochloric acid in the gastric juice in persons with nutritional macrocytic anemia and the absence of free hydrochloric acid in the gastric juice of persons with addisonian pernicious anemia even after histamine stimulation. (See Table 2.)

We have seen no cases of achrestic anemia. From a laboratory point of view the macrocytic anemias of pellagra and of pregnancy are in no way distinguishable from nutritional macrocytic anemia and all respond to folic acid. It seems wise, therefore, to abandon the terms "macrocytic anemia of pellagra" and "macrocytic anemia of pregnancy" and to classify them as nutritional macrocytic anemias. We and many others have also observed that cytologically the anemia of sprue and nutritional macrocytic anemia are identical. The relationship of the two diseases is difficult to grasp, and differentiation may not be justifiable. The final answer may have to wait until their etiology is more completely understood. Since acid steatorrhea is a characteristic and specific feature of sprue, in the presence of steatorrhea we make a diagnosis of sprue rather than of nutritional macrocytic anemia. In the Temperate Zones, only sporadic cases of so-called nontropical sprue are seen, whereas in some tropical areas sprue is endemic. There is a difference of opinion among some physicians regarding the pathogenesis of tropical and nontropical sprue, but we consider them similar conditions.

TABLE 2.—Differential Diagnosis of Some Macrocytic Anemias in Relapse

	Pernicious Anemia	Nutritional Macrocytic Anemias	Sprue	Chronic Liver Disease	Pernicious Anemia of Pregnancy	Achrestic Anemia
Dietary deficiency in animal protein	Rare	Always	Frequent	Rare	Frequent	Absent
Glossitis	Frequent	Frequent	Frequent	Rare	Frequent	Rare
Diarrhea	Frequent, alternating with constipation	Usual	Nearly always (fatty or soapy)	Rare	Rare	Rare
History or signs of pellagra, beriberi or riboflavin deficiency	Rare	Frequent	Less frequent	Rare	Frequent	Absent
Posterior lateral column degeneration	Frequent	Rare	Rare	Absent	Absent	Absent
Macrocytic anemia	Severe	Severe	Severe	Mild	Moderate to severe	Moderate to severe
Anisocytosis and poikilocytosis	Pronounced	Pronounced	Pronounced	Mild	Pronounced	Pronounced
Megaloblastic bone marrow	Present	Present	Present	Present	Present	Present
Free gastric HCl after histamine	Absent	Usual	Usual	Usual	Usual	Present
Intrinsic factor in gastric juice	Absent	Present	Present	Present	Present	Present
Response to intramuscular liver extract	Excellent	Excellent	Good	Fair	Excellent	Absent
Response to oral beef or beef extracts	Poor	Excellent	Fair (anemia)	Poor	Probably good	Absent
Response to folic acid	Excellent	Excellent	Excellent	Sometimes	Excellent	Not determined
Need for continuous specific therapy	Always	Rare	Sometimes	Rare	Rare	

TABLE 3.—MAJOR CLINICAL SYMPTOMS ON ADMISSION (CASES 1–18, TROPICAL SPRUE; CASES 19–32, ADDISONIAN PERNICIOUS ANEMIA; CASES 33–36, NUTRITIONAL MACROCYTIC ANEMIA)

			TION OF NT ILLNESS	F ITE	T LOSS, LB.	NESS	ESS	CHE	TONGUE	ITIS	IA	ING	NG OF CH	STRIC ESS	DLENCE	S HOURS	Y STOOLS	ISTENCY OOLS	T STOOLS	R OF LS	D IN LS	US IN LS	EL MOVEMENT COLIC	AL BURNING DEFECATION	OF RITY OF RHEA	RRHEA RAVATED BY FOOD	IN REMITTIES	ESTHESIAS	ING EDEMA
34	28	F	10 mo.	yes	25	ext.	sev.	sev.	no	slight	occ.	occ.	occ.	occ.	no	1	no	solid	no	brown	no	no	no	no	no diarrhea	...	yes	yes	no
35	63	F	9 yr.	yes	20	ext.	sev.	sev.	no	slight	no	no	occ.	occ.	no	1	no	solid	no	brown	no	no	no	no	no diarrhea	...	no	no	no
36	63	M	9 yr.	yes	10–15	ext.	occ.	sev.	yes	sev.	occ.	occ.	occ.	occ.	yes	3–4	yes	soft	no	brown	no	no	no	no	no particular time	...	no	no	no

*Abbreviations: ext.—extreme; occ.—occasionally; mod.—moderate; sev.—severe.

Table 3 shows briefly the major symptoms of 36 patients seen recently either in Birmingham or in Havana. Cases 1–18 are of tropical sprue, cases 19–32 are of addisonian pernicious anemia and cases 33–36 are of nutritional macrocytic anemia. Without a thorough clinical and laboratory evaluation the major symptoms such as those shown in Table 3 cannot be assessed, and without precise diagnosis the investigator in this field will be confronted with varying degrees of ignorance.

3. RESPONSE TO FOLIC ACID

The response of persons with pernicious and related anemias to synthetic folic acid (L. casei factor) has been the subject of intensive study in our clinic for over a year. Some of the results have been reported briefly; others are reported here for the first time. The results have been so tremendous and incredible that again and again I could hardly believe my eyes. The time has now come to let the evidence speak for itself (Fig. 5).

Observations have been made on 218 persons who have received folic acid. Their ages ranged from 19 to 83 years. Nine were Negroes and the rest white. The cases have been classified as follows:

78 addisonian pernicious anemia
76 tropical sprue
2 nontropical sprue
46 nutritional macrocytic anemia (pellagra)
3 macrocytic anemia of pregnancy
8 nutritional leukopenia
5 cirrhosis of liver

Every person with addisonian pernicious anemia, sprue, nutritional macrocytic anemia, macrocytic anemia of pregnancy and nutritional leukopenia has responded satisfactorily. One of the

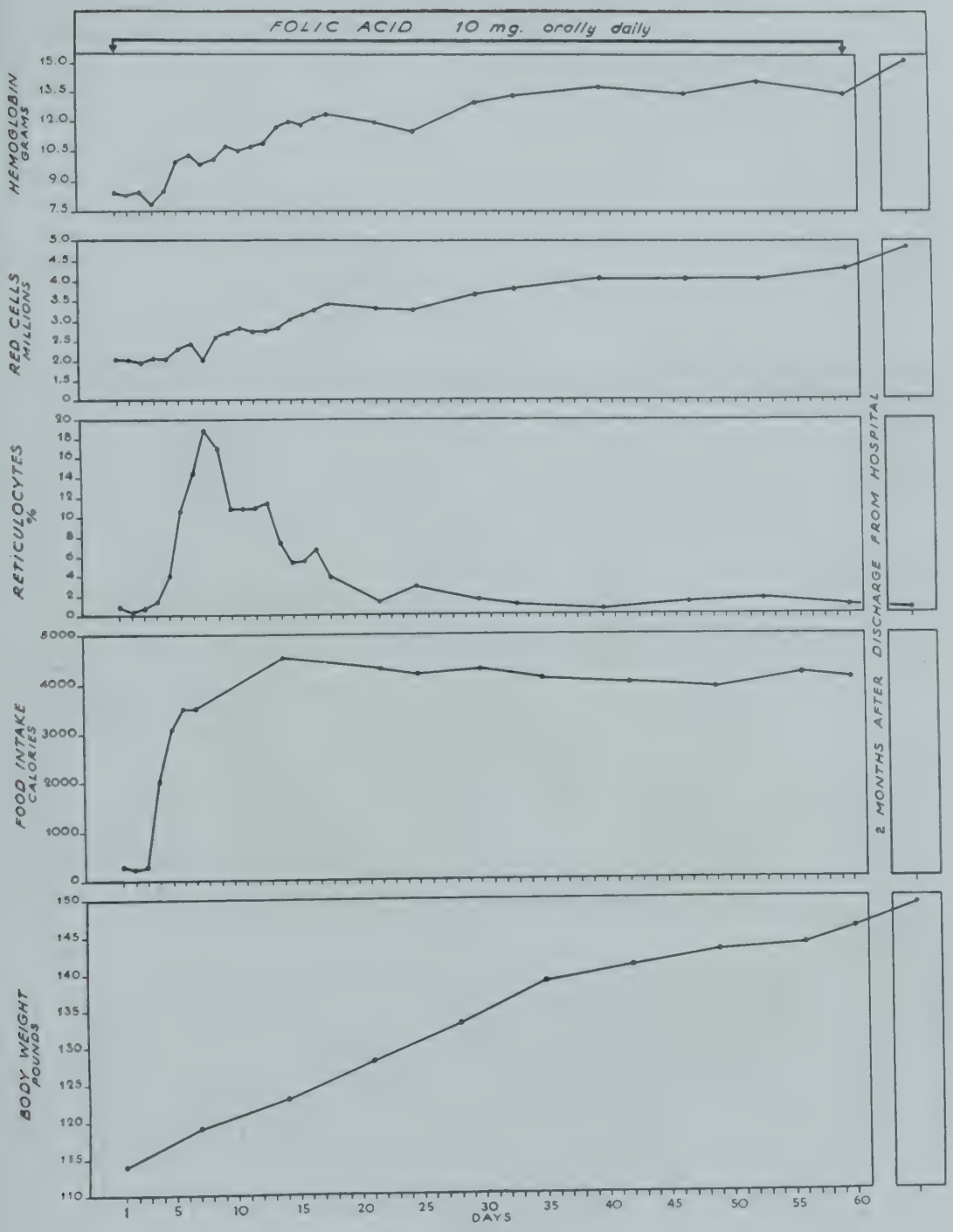


Fig. 5.—Response of patient with sprue to folic acid.

five patients with anemia associated with cirrhosis responded dramatically. The reticulocyte curve peaked at 30 per cent and there was a prompt regeneration of 1,500,000 red blood cells. In this case the level has not risen above 4,000,000. The other four patients responded poorly, if at all. The reticulocyte peaks (if they can be called peaks) were at 1–6 per cent, and although four gained 300,000 red blood cells, it is only fair to state that they have had neither a hemopoietic nor a clinical remission.

Nutritional leukopenia is to be distinguished from ordinary leukopenia and leukopenic syndromes. The leukopenia caused by aminopyrine, the sulfonamides and arsenicals does not respond to folic acid, but that accompanying megaloblastic arrest of the bone marrow usually does respond to folic acid. In the eight cases of nutritional leukopenia, the administration of folic acid was followed by an elevation of total number of circulating leukocytes and a proportionate increase in granulocytes. There was a left shift in the Arneth nuclear index accompanying the rise.

A number of physicians have asked what led me to give folic acid to patients who had pernicious anemia in relapse. They usually followed this question by asking why, once having decided to give it, I did not incubate it with normal human gastric juice. The third inquiry often is why I gave such large doses initially. I think these questions are answered in the Introduction, but it is perhaps worthy of repetition that for fifteen years I have been seeking some single pure chemical substance that would cause a hemopoietic response in such cases. For the past four years, working with Dr. L. Joe Berry and Dr. C. A. Doan, I have been studying nutritional leukopenia, which is often present in malnourished persons.[33] During a study in which we were testing the effect of a folic acid concentrate (Parke, Davis) on the leukocyte equilibrium in malnourished persons, we noted that clinical improvement followed the administration of this concentrate. This response seemed significant. Later Dr. T. H. Jukes and Dr. Stanton M. Hardy of Lederle Laboratories made available a small

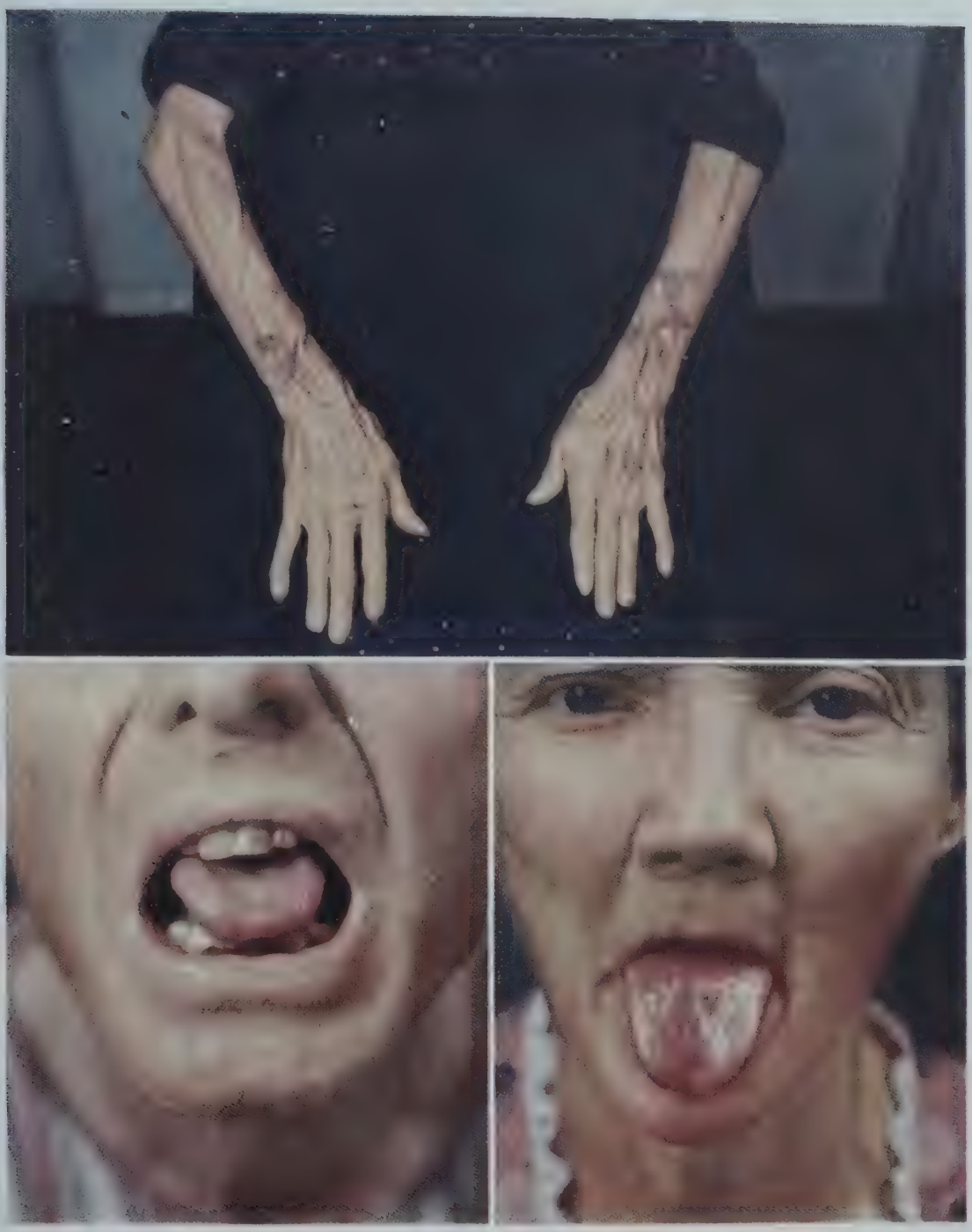


PLATE II

Fig. 1 (*above*).—Pernicious anemia. Purpura was the patient's chief complaint. After folic acid therapy the number of red and white blood cells and platelets greatly increased and the purpura disappeared.

Fig. 2 (*below left*).—Pernicious anemia. Man with slickness and atrophy of the tongue.

Fig. 3 (*below right*).—Nutritional macrocytic anemia. Woman with slickness and atrophy of the tongue.

amount of synthetic L. casei factor (folic acid) which gave similar results in five cases. This suggested that folic acid was probably an extremely important factor in red blood cell regeneration too, but scarcity of the material made adequate testing difficult. When Dr. Jukes made more of this synthetic substance available it was immediately tested in nine cases of macrocytic anemia in relapse.[34] The material was dissolved in saline made slightly alkaline with small amounts of sodium bicarbonate and injected intravenously each day in five cases. This was followed in a few days by reticulocytosis and an increase in hemoglobin and red blood cell level. By this time more synthetic material had been obtained and the other four patients were given it daily by mouth. Since the optimal dose was not known, 50 mg. was given twice a day to some of the patients and 50 mg. three times a day to others. All responded satisfactorily. They looked and felt better and the blood values increased.

Following these observations, I communicated the findings to Dr. C. A. Doan and Dr. Carl Moore, both of whom had previously made scientific contributions to the Clinic of momentous significance. Before preparing a report for publication, I was concerned about the best manner of presenting our results. I was aware that many physicians did not have all the data at hand and would be harsh in their criticism if we took a long step forward and considered synthetic folic acid to be an antianemic factor in its own right. Nevertheless, I decided to state that it was an antianemic factor effective in certain types of macrocytic anemias and to make no claim that it was the sole antipernicious anemia factor of liver, which I knew it was not, and to make no claim that it was the extrinsic factor of Castle, which I did not believe, but to state merely that folic acid itself was an antianemic factor capable of promoting regeneration of the blood cells and hemoglobin in properly selected cases.

In the next paper Vilter, Spies and Koch[35] reported that folic acid gave effective relief in six cases of nutritional macrocytic

anemia, five of addisonian pernicious anemia and three which were classified as indeterminate since there was some difference of opinion among the physicians as to whether they should be considered as sprue, pernicious anemia, nutritional macrocytic anemia or the macrocytic anemia of pellagra. The status of the gastric contents after histamine-phosphate stimulation is shown in Table 4.

TABLE 4.—Status of Gastric Contents After Histamine-Phosphate Stimulation

Case 1.	Achlorhydria and no pepsinogen 1944, 1945; rennin absent in 1944, but present in 1945
Case 2.	Achylia 1940, 1943, 1944
Case 3.	Achylia 1941, 1942, 1943, 1944; achlorhydria in 1945, and no rennin; pepsinogen in 1945
Case 4.	Achlorhydria and no rennin 1944; pepsinogen in 1944
Case 5.	Achylia 1945
Case 6.	Achylia 1945
Case 7.	Free acid 1937, 1940, 1944; achlorhydria 1936, 1938, 1943, 1945; rennin and pepsinogen 1945
Case 8.	Achylia 1944; achylia on one occasion in 1945; pepsinogen present on one occasion
Case 9.	Achylia 1944, 1945
Case 10.	Achlorhydria 1943, 1944, 1945; pepsinogen 1944, 1945; rennin 1944, 1945
Case 11.	Achylia 1943, 1944, 1945
Case 12.	Free acid and enzymes 1945
Case 13.	Achlorhydria twice in 1945; no rennin; pepsinogen present
Case 14.	Achylia 1945

In this group also the diet was closely regulated so that chances of a spontaneous remission would be minimal. The effect of folic acid on the red blood cell and hemoglobin degeneration was similar to that previously reported. The clinical changes were again dramatic. A feeling of subjective improvement occurred between the third and the fifth day, usually slightly before the day of the initial reticulocyte response. In both groups, when diarrhea was a prominent feature, the stools reverted to normal within 48 hours. The burning of the tongue and the glossitis disappeared. A ravenous appetite accompanied the onset of the remission. Consistent with the usual pictures of pernicious anemia, nutritional macrocytic anemia and nutritional leukopenia, the white blood

cell counts were low, but the level increased following therapy, as shown in Table 5.

Thus the striking hematologic response was again evident. The increase of reticulocytes and the rise in red blood cell, hemoglobin, white blood cell and platelet contents were definite. When treatment was continued, the regeneration proceeded to normal levels (Figs. 6 and 7). Erythrogenesis occurred regardless

TABLE 5.—Effect of Folic Acid on Leukopenia Associated with Macrocytic Anemia

Case	White Blood Cells				Route of Administration
	Initial	7th Day	14th Day	21st Day	
1-B	5,150	6,400	3,800	9,350	I.M.
3	3,100	2,850	6,050	4,500	I.V. and oral
4	2,850	5,500	3,250	4,000	I.V.
5	2,000	1,900	3,550	3,550	I.V.
6	7,150	5,400	4,550	6,800	oral
7	6,200	5,450	6,250	8,700	oral
8	4,300	5,750	8,650		oral
9	6,050	4,400	8,700	9,850	oral
10	2,950	3,500	7,550	8,350	oral
11	6,550	6,100	6,750	7,800	oral
12	4,650	4,050	8,800	8,050	oral
13	5,100	6,000	10,800	14,850	oral

of the clinical classification of the macrocytic anemia. It was stressed that the response to treatment paralleled that obtained with potent liver extract, although the red blood cell regeneration was not quite as rapid as would be expected from a maximal dose of potent liver extract. It was stated also that folic acid probably would not be more effective in these conditions than liver extract given in large amounts.

Moore, Bierbaum, Welch and Wright[55] also obtained clinical and hematologic remissions in two patients with addisonian pernicious anemia following the administration of synthetic L. casei factor. One patient received a daily oral dose of 100 mg. for 10 days. The initial red cell count of 1,200,000 rose to 3,000,000 beginning about the seventh day of therapy and was constant for

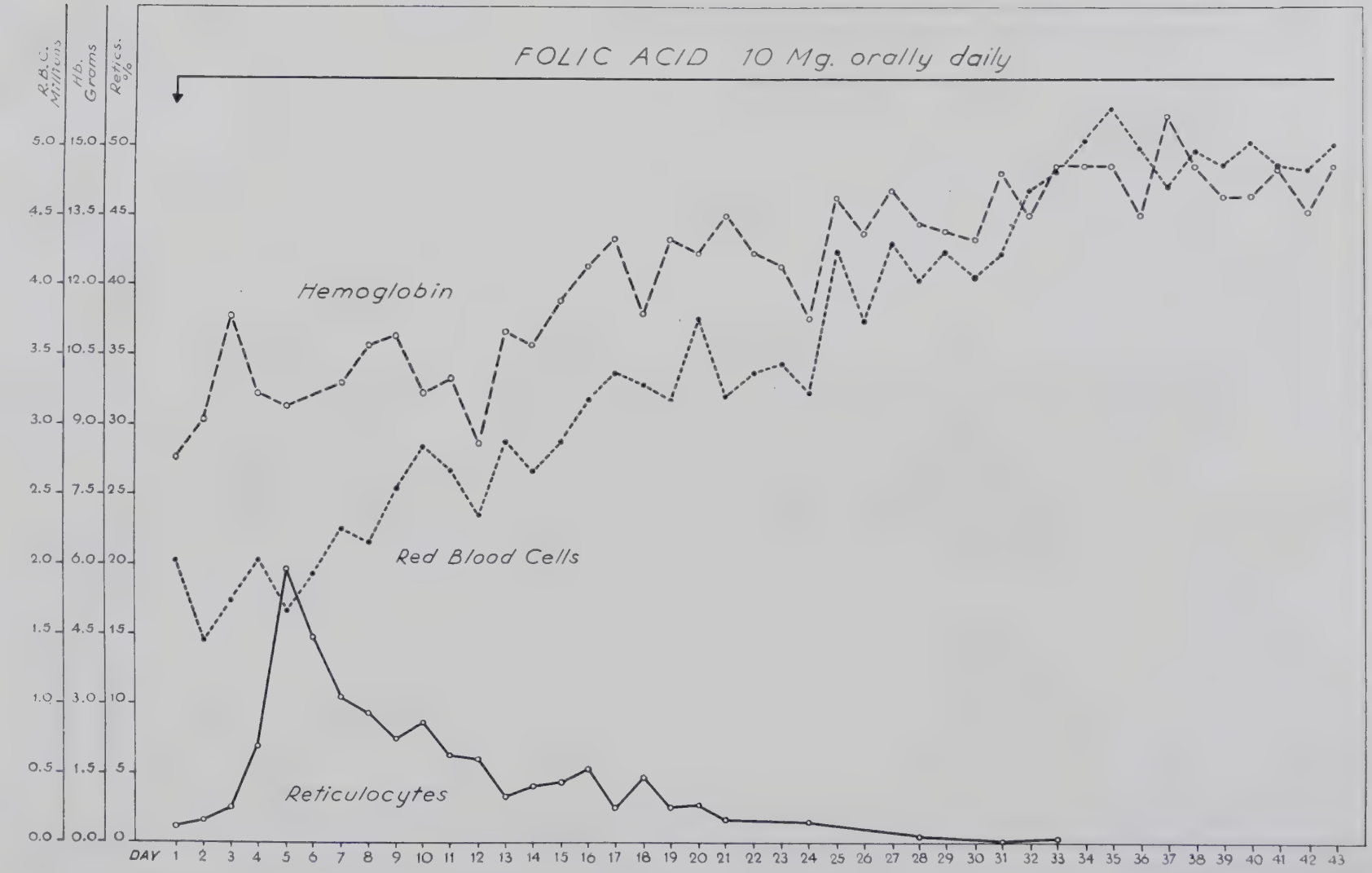


Fig. 6.—Response of patient with pernicious anemia to folic acid.

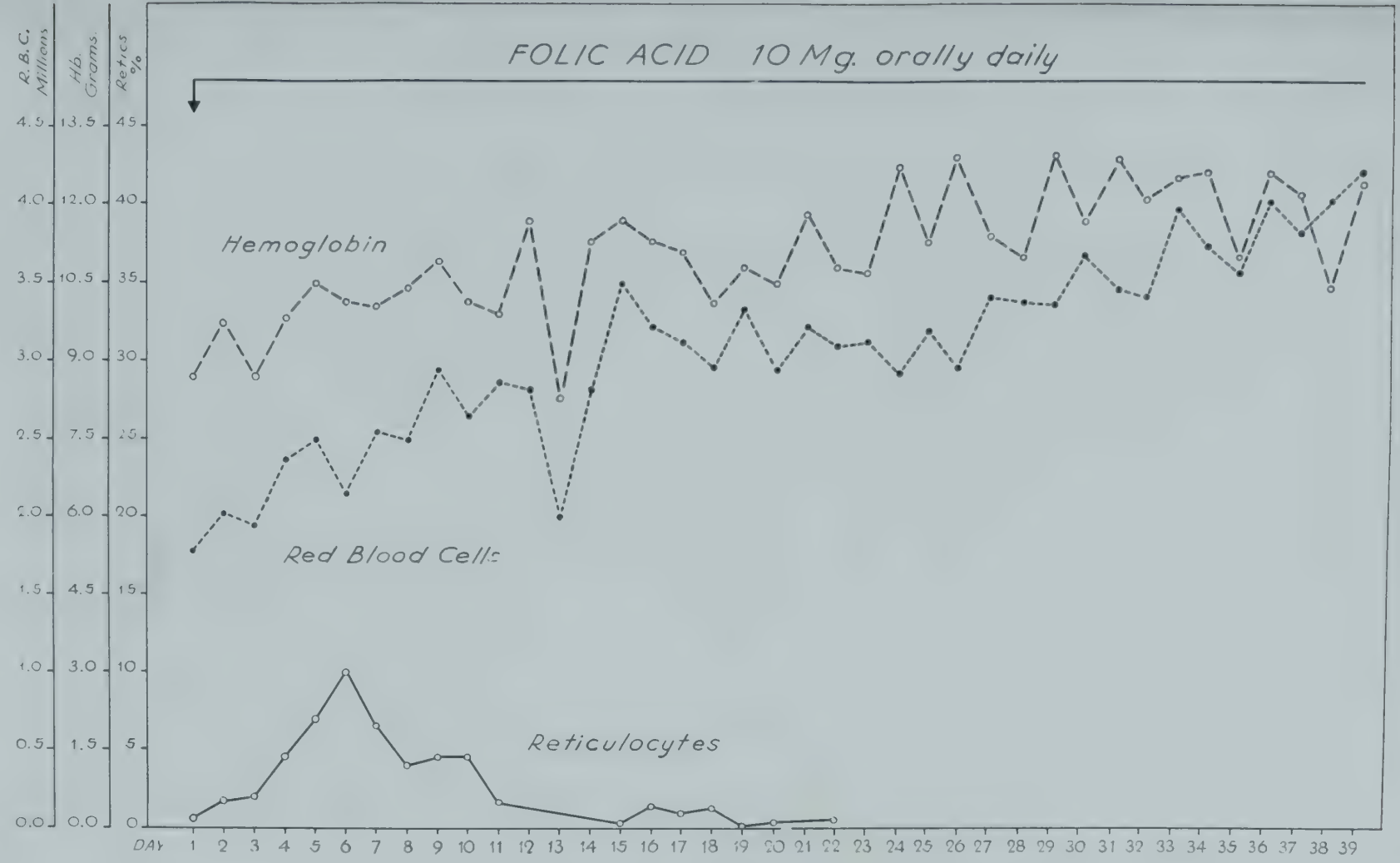


Fig. 7.—Response of patient with nutritional macrocytic anemia to folic acid.

four weeks. The reticulocytes peaked at 40 per cent on the seventh day. The second patient had 700,000–950,000 red blood cells, increased by transfusion to 1,400,000–1,500,000, following which an oral dose of 30 mg. of folic acid was given daily for 14 days. A peak of 44.5 per cent for reticulocytes was reached on the eighth day; the red blood cell count began to rise on the sixth day and reached 2,500,000 by the fourteenth day. The leukopenia and thrombocytopenia disappeared. One patient with nontropical sprue was given 20 mg. of L. casei factor parenterally per day for 10 days and 40 mg. every two days for two weeks. The initial erythrocyte count of 2,600,000 rose to 3,500,000 and leveled off after 12–14 days. The maximum reticulocyte response of 30.2 per cent occurred on the seventh day. Leukocyte and platelet values became normal within seven to 10 days. A patient with pernicious anemia of pregnancy was given 20 mg. of folic acid parenterally daily for 10 days. The erythrocyte count increased from 1,100,000 to 3,000,000 in 15 days, and 48.2 per cent reticulocytes were found on the seventh day.

Late in 1945 Darby and Jones[56] presented a preliminary report on the parenteral treatment of nontropical sprue with synthetic L. casei factor. One case was followed for 15 days and the second for four days. Reticulocytosis occurred in both.

Spies, Garcia Lopez, Menendez, Minnich and Koch[36] reported that patients with tropical sprue in Cuba had dramatic hemopoietic response accompanied by a definite upsurge of well-being. Diet histories* showed that all the patients had subsisted on diets of rice, bread, potatoes, viandes and bean soup. They never had meat, poultry or fish. They were pale, weak and disinterested in their surroundings. They had severe diarrhea, characterized by copious, frequent, watery stools which were yellow or white. During the preliminary period of observation they refused everything but coffee. About the fifth day after treatment they became generally interested in their surroundings. They became able to

* Obtained by Mrs. Myrtle Neblett and Miss Jean Grant.

walk around the ward, and the stools tended to become more normal. Table 6 shows the early response of the first three patients with tropical sprue treated with folic acid. In such cases, when therapy is continued the blood values improve to normal (Fig. 8). Associated with this hemopoietic response is a striking gain in strength, vigor and appetite.

More extensive reports definitely established the value of synthetic L. casei factor in certain clinical types of macrocytic anemia.[57,58] Remissions were obtained in five cases of nutritional macrocytic anemia, five of addisonian pernicious anemia, eight

TABLE 6.—Response of Sprue to Oral Administration of Folic Acid

Case	Red Cells, Millions		Hemoglobin, Gm.		Reticulocytosis		
	Initial	10th Day	Initial	10th Day	First Day of Rise	Day of Peak	% at Peak
1	2.11	2.95	9.0	11.1	5	7	17.2
2	2.16	2.66	9.5	10.3	5	7	17.2
3	1.15	2.27	6.6	8.0	4	6	22.7

of sprue, three of macrocytic anemia of pregnancy, one of macrocytic anemia associated with chronic alcoholism, one of cirrhosis of the liver, one of neuritis, one associated with carcinoma of the stomach and three of indeterminate etiology. No response to folic acid was detected in three cases of aplastic anemia, three of leukemia and four of iron deficiency anemia. (Since then the failure of folic acid has been observed in eight patients with hypochromic anemia, six with leukemia, seven with aplastic anemia, one with idiopathic leukopenia, 16 with drug idiosyncrasies or postinfectious leukopenia and four with idiopathic steatorrhea.) Daily oral doses as large as 400 mg. were given without ill effects.

More extensive studies of tropical sprue were then made with Milanes, Menendez, Koch and Minnich[59] in Cuba and with Ramon M. Suarez, Ramon M. Suarez, Jr., and Hernandez-Morales[60] in Puerto Rico. The dietary history in each of 14 cases showed

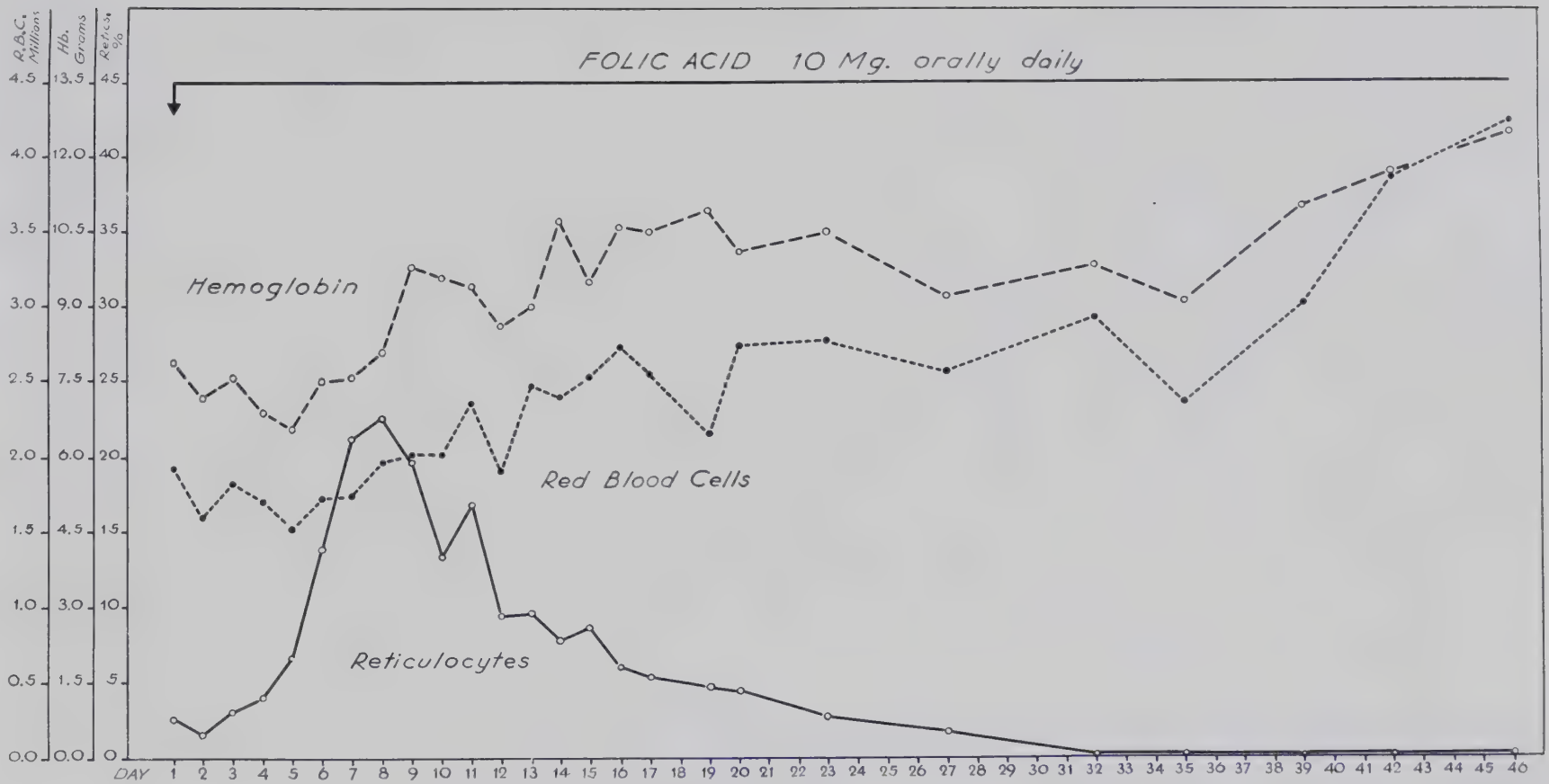


Fig. 8.—Response of patient with sprue to folic acid.

that the diet had been deficient in animal protein over a long period and had consisted almost entirely of rice, corn meal and root vegetables. All patients had lost weight. All gave a history of diarrhea for periods ranging from four months to five years. The stools were light-colored, large and frothy and varied in number from three to 20 a day. Nine of the 14 patients had dependent pitting edema. All the patients had macrocytic anemia with mean corpuscular volume of from 105 to 145 cubic microns and

TABLE 7.—Antianemic Effect of Folic Acid in Tropical Sprue

Case	REC, Millions		Hbg., Gm.		Reticulocytes, %			Dosage of Folic Acid			Diet
	1st Day	Final Day	1st Day	Final Day	1st Day of Rise	Day of Peak	% at Peak	Daily Dose, Mg.	No. of Days	Total, Mg.	
1	0.6	2.25	2.4	7.5	4	5	18	50	18	900	No meat products or yeast
2	1.0	2.02	4.9	6.9	5	6	25.4	50	14	700	
3	1.7	2.48	8.4	10.1	3	5	17.0	50	14	700	
4	2.3	2.5	10.1	11.0	3	4	7.8	50	12	600	
5	2.1	2.7	9.9	10.1	4	7	21.2	50	8	400	Meat allowed

mean corpuscular hemoglobin of from 34 to 47 micromicrograms. The erythrocyte counts ranged from 600,000 to 2,300,000 per cu. mm. The red blood cells showed marked anisocytosis, poikilocytosis and polychromasia. Free hydrochloric acid was present in the gastric contents of all the patients. During the test period, with one exception, the diets of all the patients were limited to contain no meat, meat products, poultry or fish. Such restriction of diet is not recommended in the treatment of sprue; rather, a diet high in vitamins and protein is important to hasten recovery. An idea of the response of the peripheral blood and bone marrow can be obtained in Tables 7 and 8. Folic acid had

TABLE 8.—Differential Bone Marrow Counts in Tropical Sprue

Case	Basophils, %	Eosinophils, %	Myelocytes, %	Metamyelocytes, %	Band Cells, %	Segmented Cells, %	Lymphocytes, %	Clasmatocytes, %	Megakaryocytes, %	Plasma Cells, %	Reticulum Cells, %	Normoblasts per 100 WBC	Late Erythroblasts per 100 WBC	Early Erythroblasts per 100 WBC	Megaloblasts per 100 WBC	Nucleated RBC per 100 WBC
1	0	3	C-27 C-22	20	19	18	8	3	1	1	0	6	19	15	2	42
2	0	3	B- 2 C-26.5	18	10	10	33	1	0	1	0	25	12	8	2	47
3	0	2.5	B- 1 C-17	15.5	12.5	23.5	16	1.5	1	0	0	15	18	16	5	54
4	0	2.5	B- 0.5 C-37	17	7.5	32.5	20.5	0.5	0	0	2	28	24.5	29.0	15.5	97
5	0	1	B- 2 C-13.5	28	7	9	14	3	1	0	0	23	10	16	11	60
6	0	6	B- 1.5	17	5	30	23.5	3.5	0	0	0	12	35	17	3	67
7	0	1	C-27 C-17	28	11	10	20	0	1	1	0	12	22	7	0	41
8	0	3	B- 3	17	17	26	13	1	1	2	0	4	12	15	4	40
9	0	2	C-10	10	10	49	17	1	1	0	0	34	17	4	4	59
After Folic Acid Administration																
1	0	1	C-25	12	14	25	17	5	1	0	0	71	13	3	0	87
8	0	13	C-12 C-18	8	16	31	18	1	1	0	0	109	7	5	0	121
9	0	1	B- 1	8	10	26	32	2	1	0	1	117	4	2	0	123

a profound effect on the alimentary tract of these persons. The number of stools per day decreased, and they became better formed and more normal in appearance.

Doan, Wilson and Wright[61] showed that as little as 2 micrograms of synthetic folic acid per day, when administered parenterally for 20 days, produced the maximum calculated reticulocyte crisis. This was followed by an increase in red blood cell and hemoglobin values and a reversal of the bone marrow activity. These authors also satisfactorily and safely substituted folic acid for liver extract in persons who had developed sensitivity to liver extract.

Zuelzer and Ogden[62] and Zuelzer[63] have reported that folic acid is an antianemic factor specifically effective in anemia of infancy associated with megaloblastic bone marrow. They reported complete parallelism in the response to folic acid by this type of anemia of infants and the types which I have reported in adults. In their follow-up studies 10 months after therapy they observed no relapse.

The clinical evidence in support of folic acid as a hemopoietic factor is rapidly accumulating.[64-69] The author and his Cuban associates have made unpublished observations of complete rehabilitation of 18 patients with tropical sprue. All have been discharged with essentially normal blood values. Fifteen went back to work. Three, who are over 70 years old, had retired before their illness and after onset had been a burden to their families; they are now able to care for themselves.

All the patients under 60 who had uncomplicated nutritional macrocytic anemia or pernicious anemia and who have been treated fully have also been rehabilitated. A number over 60 are not working but are no longer dependent on their families for personal care.

In our series of cases is a considerable number of patients whose red blood cells could not be brought to a full 5,000,000. In every case in which blood values failed to rise to this desired level,

liver extract was given subsequently in large doses, but in no instance did it cause additional blood regeneration. These patients were then given all the known synthetic vitamins, and they too caused no further blood regeneration. Eight patients with macrocytic anemia had a definite iron deficiency, and it was necessary to give them iron before their red blood cell values could be brought to normal. In some patients reticulogen increases the red blood cell values a little more rapidly than the dosage of folic acid we have used, but apparently it does not bring them to a higher level. Recently another antianemic substance, 5-methyl uracil (thymine), has proved effective when given in large doses (Figs. 9 and 10). The bone marrow and peripheral blood responses parallel, but at a much suppressed level, those to potent liver extract and synthetic folic acid.

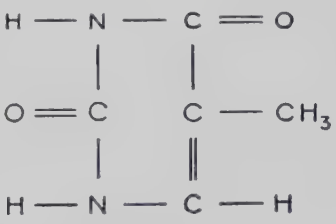


Fig. 9.—Thymine.

Patients maintained on synthetic thymine almost always show a progression of glossitis and stomatitis. Thymine is a constituent of nucleic acid. Since nucleic acids have long been known to play an important role in cellular metabolism, there is a suggestion that antianemic factors are linked in some way to nucleic acid synthesis. There is little doubt that liver extracts contain antianemic principles which are different from those of folic acid and which could not possibly contain sufficient thymine to bring about a remission. Thus Castle's erythrocyte maturation factor must be either the end-product of the action of these several substances or else several different substances capable of leading to the same end-result. Should this end-result be bound in with

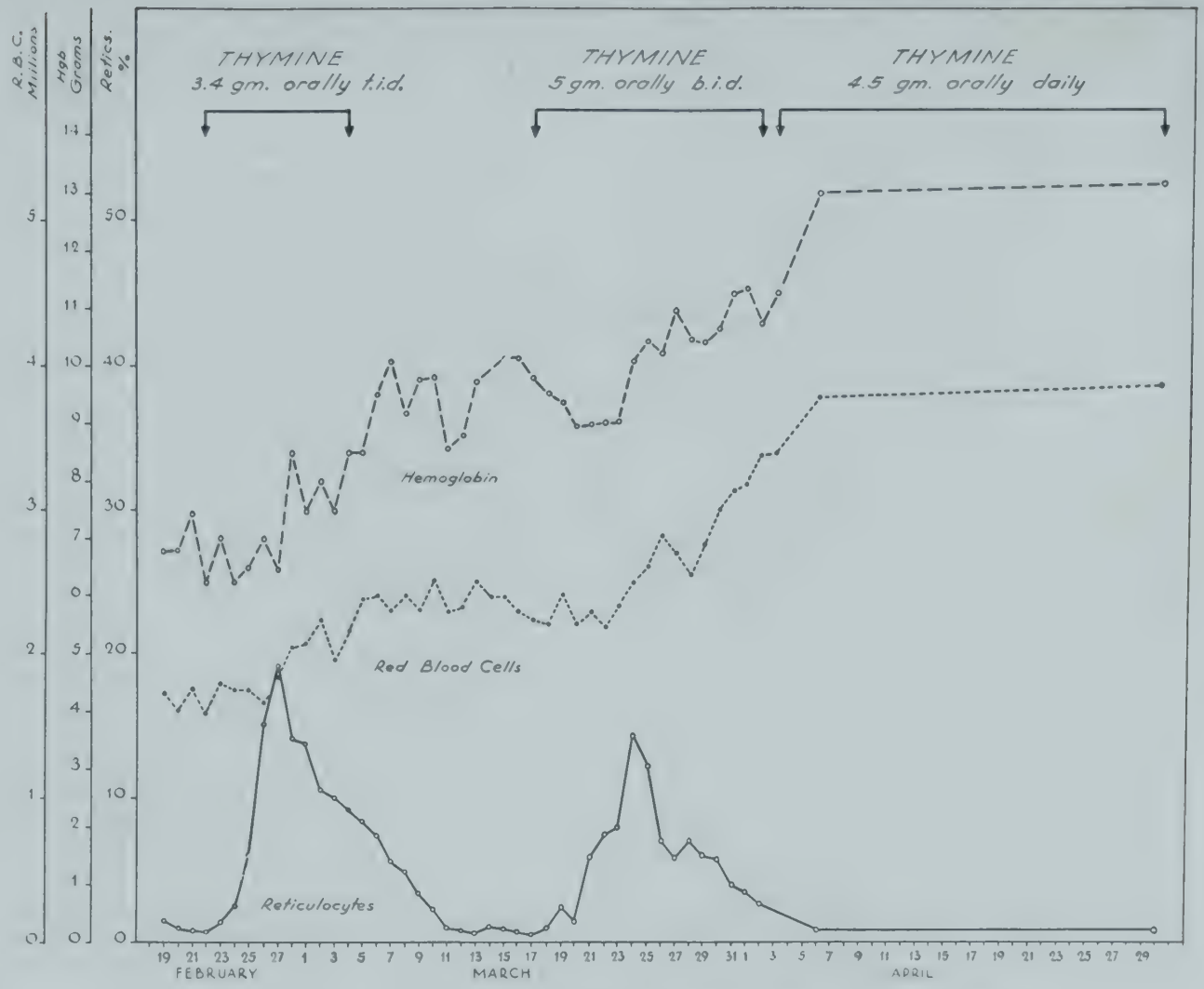


Fig. 10.—Response to thymine of a white woman, aged 57, with histamine-refractory achlorhydria and achylia.

nucleic acid formation, it would seem, a priori, that folic acid and other antianemic factors might act in a similar over-all manner but by different routes.

Many years of careful study of persons with deficiency states have led to the conviction that certain fundamental biochemical systems in the human body do not function perfectly in the presence of clinical deficiency states. Until more specific data can be accumulated regarding these biochemical systems and the biochemistry of the antianemic compounds, it might be wise to avoid unfounded speculation. Certain facts, however, should be stressed. The failure of patients to respond to the oral administration of 10 Gm. of synthetic uracil (Fig. 11) is of great interest

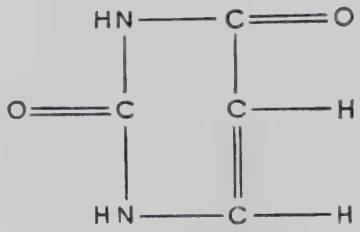


Fig. 11.—Uracil.

in the light of their subsequent response to a similar dose of 5-methyl uracil (thymine). This indicates a great specificity of the 5-methyl group of the thymine molecule. The same patients, who received uracil and 5-methyl uracil during another relapse, responded maximally to the oral administration of 10 mg. of folic acid per day. Dr. Walter Frommeyer and I studied the relative response of the same persons, in different periods of relapse, to reticulogen, folic acid and thymine (Fig. 12) and clearly demonstrated that 5-methyl uracil is of great scientific interest and of little practical therapeutic importance. When 15 Gm. of thymine was given orally per day, the rate of red blood cell rise and the peak of reticulocytes were of a lower order than those which follow either the daily intramuscular injection of 0.5 cc. of reticulogen or the oral administration of 20 mg. of folic acid. Dr. Frommeyer,

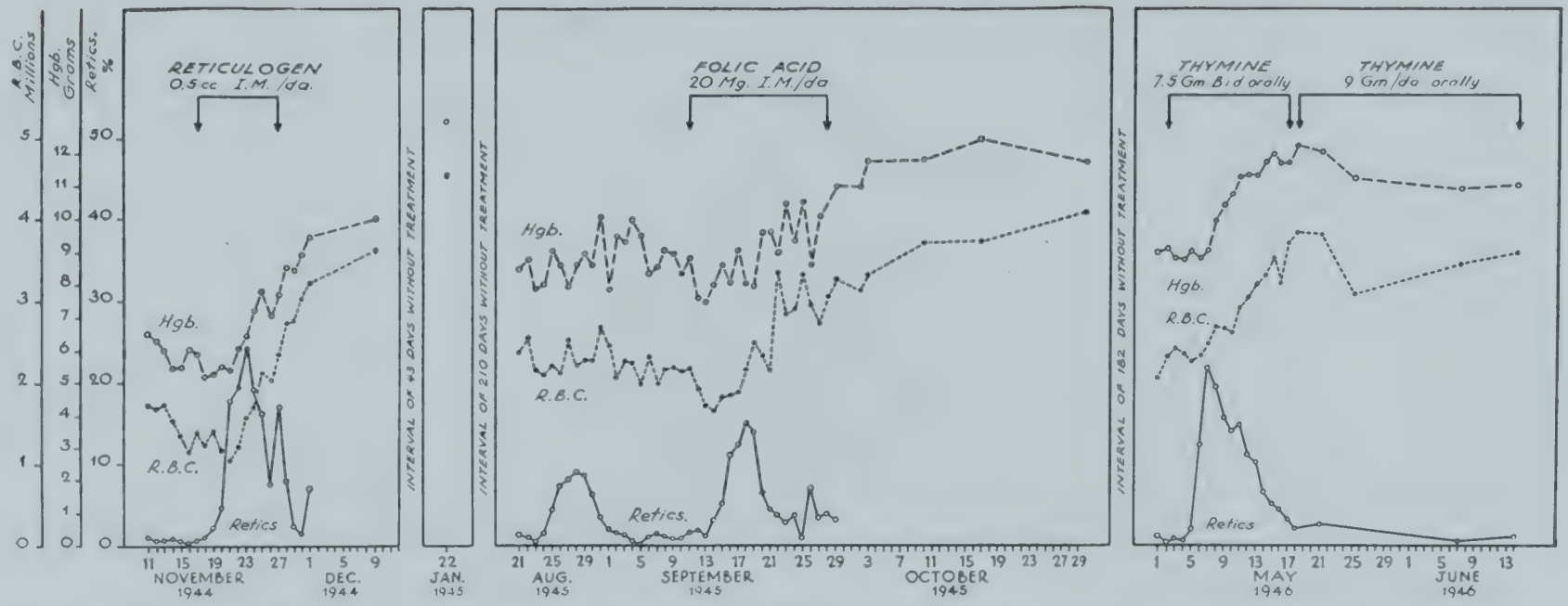


Fig. 12.—Response to reticulogen, folic acid and thymine of a white man, aged 66, with histamine-refractory achlorhydria and achylia.

Dr. Jose Aristides Menendez and I found guanine (Fig. 13), adenine (Fig. 14) and pyridoxine (Fig. 15) ineffective in patients who later specifically responded to 10 mg. of folic acid each day.

Recently I found that the naturally occurring fermentation L. casei factor produces a remission in addisonian pernicious anemia.[70] The fermentation L. casei factor differs from the L. casei factor, which was originally isolated from liver and which

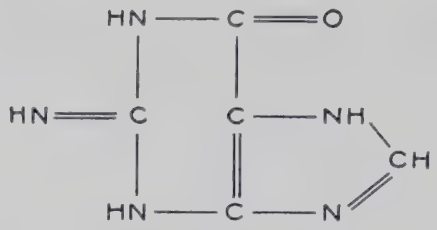


Fig. 13.—Guanine.

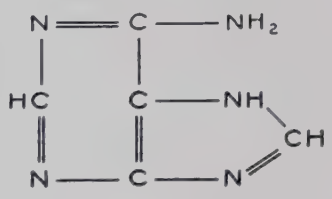


Fig. 14.—Adenine.

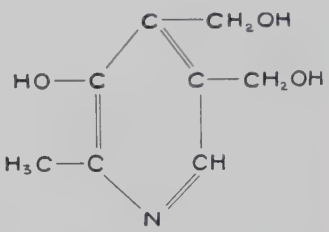


Fig. 15.—Pyridoxine.

has since been synthesized,[71] in having an additional two molecules of glutamic acid, as can be seen by comparing the two formulas (Figs. 16 and 17). The chemists probably will call the L. casei factor pteroylglutamic acid. The vitamin B_c conjugate, which was isolated by Pfiffner and his associates[24] and which the chemists probably will call pteroyl hexa-glutamyl glutamic acid, has a molecular weight 2.8 times that of the liver L. casei factor. Chemically it is the same except that it has six molecules of glutamic acid attached. Dr. Robert E. Stone and I have obtained a hemopoietic response in patients with addisonian pernicious anemia and with nutritional macrocytic anemia following the administration of 14 mg. of this compound. Sharp[78] has obtained

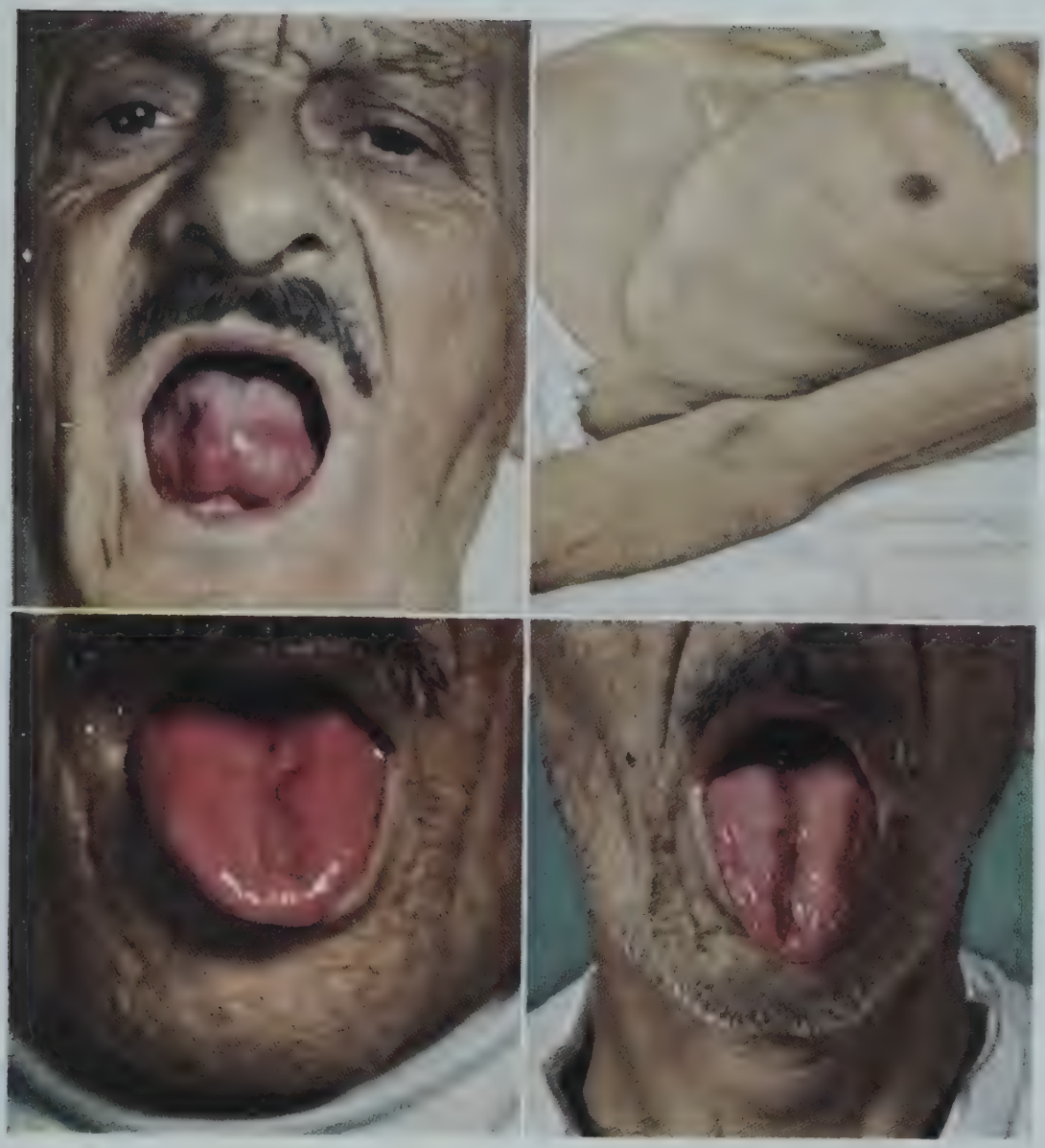


PLATE III

Fig. 1 (*above left*).—Tropical sprue. Man with glossitis on the tip and lateral parts of the tongue. This disappeared with general improvement.

Fig. 2 (*above right*).—Tropical sprue. Note pallor, extreme emaciation of the abdomen and purpura of the antecubital fossa.

Fig. 3 (*below left*).—Tropical sprue. Glossitis characterized by slickness and fiery redness.

Fig. 4 (*below right*).—Same patient after two weeks of folic acid therapy. The redness is greatly diminished; the slickness remains. In most cases, regeneration of the papillae is rapid.

similar results. Failure to obtain a hemopoietic response to the vitamin B_c conjugate, reported by Bethell, Swendseid, Bird, Meyers, Andrews and Brown[79] and by Heinle, Nelson, Nelson and

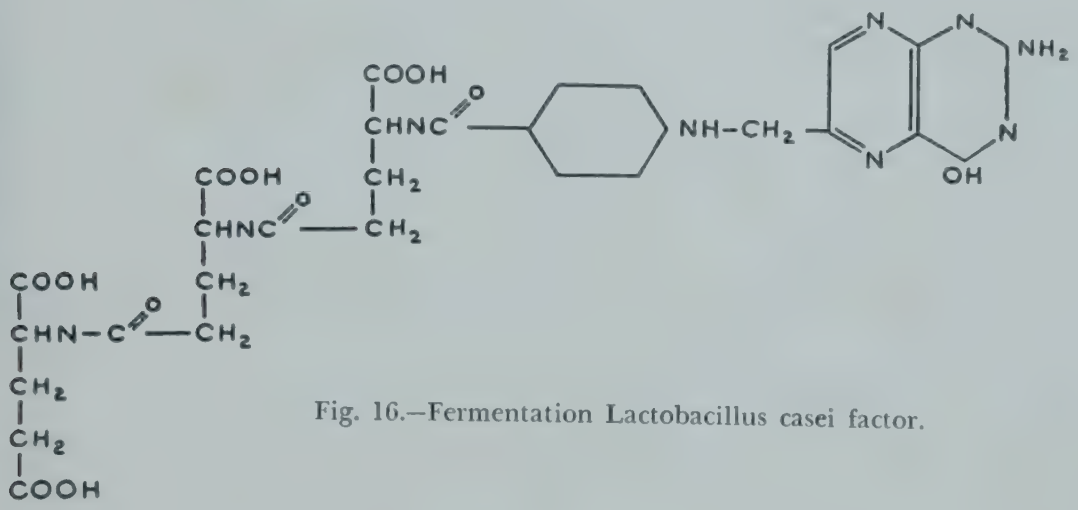


Fig. 16.—Fermentation Lactobacillus casei factor.

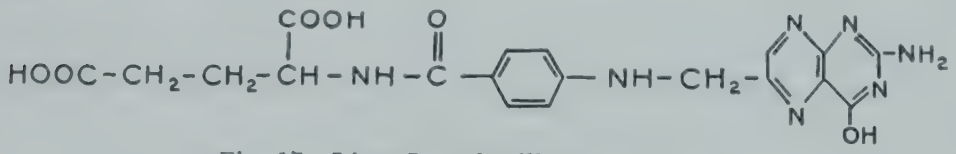


Fig. 17.—Liver Lactobacillus casei factor.

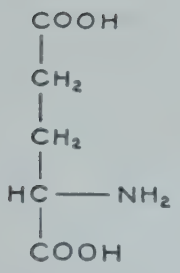


Fig. 18.—Glutamic acid.

Fig. 19.—Para-aminobenzoic acid.

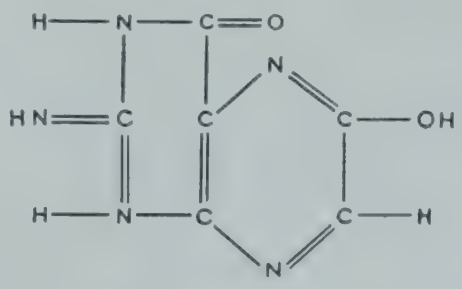


Fig. 20.—Xanthopterin.

Welch,[80] may have been due to their having given insufficient amounts. Since the L. casei factor contains glutamic acid, para-aminobenzoic acid and a pterdyl ring, we have given glutamic acid (Fig. 18), para-aminobenzoic acid (Fig. 19) and xanthopterin (Fig. 20) in the following daily oral doses: 5 Gm., 5 Gm. and 500

mg., respectively, for 10 days. No blood regeneration was detected, but the same patients responded spectacularly to the oral administration of 10 mg. of folic acid (liver L. casei factor). These observations indicate that the patients' bodies could not efficiently, if at all, unite these components to form folic acid and thus induce a remission. In a selected case of addisonian pernicious anemia in relapse, 5 mg. of pteroic acid, which is that part of the L. casei factor devoid of glutamic acid (Fig. 21), injected intra-

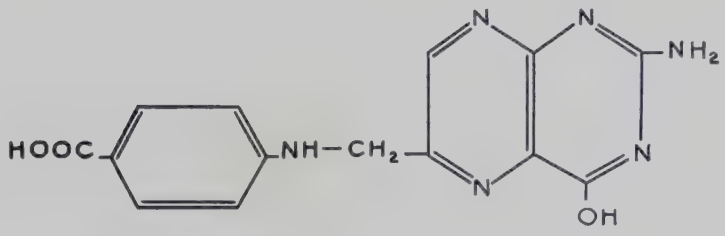


Fig. 21.—Pteroic acid.

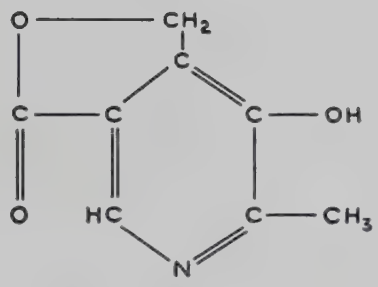


Fig. 22*A*.—Alpha pyracin.

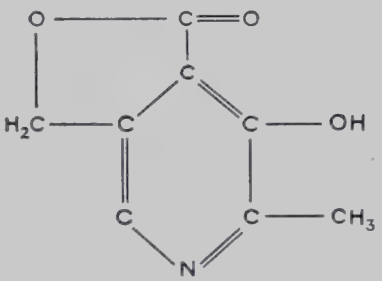


Fig. 22*B*.—Beta pyracin.

muscularly daily for 10 days caused no response. The same patient was then given 5 mg. of liver L. casei factor (folic acid), to which he responded maximally. Daily oral doses of 100 mg. of alpha pyracin (Fig. 22*A*) and of beta pyracin (Fig. 22*B*) were ineffective.

At this point I should like to emphasize that at least four crystalline compounds with similar physiologic properties, yet differing in some respects, have been obtained from liver, yeast or other sources. We already know the chemical construction of

three, and that of others will soon be known. Then we shall be able to determine better the effect of each on the cells of the bone marrow. In the meantime it is well to keep in mind that although I have included pernicious anemia in the group of macrocytic anemias which respond to folic acid, I realize that its natural pathogenesis is somewhat different from that of macrocytic anemia of sprue, of pregnancy and of pellagra and from that of nutritional macrocytic anemia. I believe that folic acid functions as a part of an enzyme system and that this same fundamental system is disturbed in all these diseases. From the onset I have stressed, as has Moore, that no satisfactory explanation has been made for the fact that, to produce a satisfactory hemopoietic response, a relatively large amount of folic acid is required as compared with liver extract, in which the amount of active substance is much smaller. From our studies it would seem that the active principle in liver extract is not folic acid or any of the chemical substances mentioned in this monograph but is a more powerful substance which, when obtained in pure form, will be more efficacious per unit of weight than any now available. Could it be that folic acid conjugates are stored in our bodies and that liver extract contains substances capable of liberating them so that they can act on the cells of the bone marrow?

As already noted, the profound improvement of alimentary tract function following the administration of folic acid led my Cuban colleagues and me to study in great detail some of these changes. The results of the examinations are summarized in Tables 9, 10 and 11.

We observed a conspicuous absence of parasites in samples obtained by curettage from the rectum and sigmoid. Intestinal parasitic ova, cysts or adult forms were present in the feces of 56 per cent of the patients and in the contents of the small intestine in 12 per cent. Bacterial culture revealed a multiplicity of organisms, most of which are normal intestinal flora. In the feces of 72 per cent of the cases was observed a gram-positive, nonsporulat-

TABLE 9.—Parasites, Yeasts and Bacteria in Feces*

Case	Age	Sex	Parasites	Yeasts	Bacteria	pH
1	29	F	Trichuris trichiura (ova)	neg.	Staphylococcus; Enterococcus; Butyribacterium	6.0
2	45	F	do.	do.	Es.coli;Butyribacterium	4.5
3	48	F	T. trichiura; Ascaris lumbricoides (ova)	do.	Es. coli; Es. acidi lactici; Butyribacterium	7.5
4	67	F	neg.	do.	B. proteus; Staphylococcus; Es. coli	6.0
5	31	F	T. trichiura	Monilia	Enterococcus; Es. coli; Butyribacterium	
6	68	F	T. trichiura (ova)	neg.	Es. acidi lactici; Butyribacterium	4.5
7	33	F	neg.	do.	Es. coli; Aerobacter	5.0
8	52	M	T. trichiura (ova)	Monilia	Es.coli;Butyribacterium	4.5
9	40	M	neg.	neg.	Es. coli; Es. acidi lactici; B. proteus; Butyribacterium	5.5
10	70	M	neg.	Hypho-mycetes	B. proteus	7.5
11	63	M	Es. nana; Es. coli (cysts)	neg.	Es. coli; Es. acidi lactici; Butyribacterium	5.5
12	44	M	neg.	Hypho-mycetes; Monilia	Es. coli; Enterococcus; B. proteus; Butyribacterium	4.5
13	63	M	Trichuris; Necator americanus; Strongyloides stercoralis (ova and larvae)	Monilia	Butyribacterium	6.0
14	43	M	neg.	Blastocystis hominis	Pseudomonas; Enterococcus; Es. coli; Butyribacterium	4.5
15	75	M	Es. coli (cyst); A. lumbricoides (ova)	neg.	B. pyocyaneus; Enterococcus; Alkaligenes; Staphylococcus; Es. coli; Butyribacterium	6.5
16	65	M	Trichuris; Necator (ova)	do.	Es. coli; Es. acidi lactici; Butyribacterium	5.5
17	63	M	neg.	Monilia	Klebsiella pneumoniae; Butyribacterium	4.5
18	62	M	Trichuris (ova)	neg.	Es. coli; B. proteus; Butyribacterium	7.0
19	37	M	Trichuris; Necator (ova)	do.	B. proteus; Butyribacterium	7.5
20	66	M	Trichuris; Necator; Giardia (ova & veg. form)	do.	Es. coli; Enterococcus; Butyribacterium	4.5
21	46	M	neg.	B. hominis	neg.	6.5
22	24	M	do.	Monilia	Es. coli; Staphylococcus; Butyribacterium	5.5
23	77	M	Trichuris; Necator (ova); Chilomastix (cyst)	B. hominis	B. proteus; Es. coli; paracolon bacillus	5.5
24	41	M	neg.	pos.	B. proteus; Es. acidi lactici	7.5
25	70	M	do.	neg.	Es. acidi lactici; Enterococcus	6.0

*Typical in 23 cases.

TABLE 10.—Observations on Material Obtained by Rectosigmoidoscopy*

Case	Age	Sex	Mucosa	Bacteria
1	29	F	pale, hypotrophic	neg.
2	45	F	do.	B. proteus
3	48	F	pale, hypotrophic, slight congestion	Enterococcus; Es. acidi lactici
4	67	F	pale, hypotrophic	B. proteus; Staphylococcus
5	31	F	do.	Es. acidi lactici; Staphylococcus
6	68	F	do.	neg. (mucous material)
7	33	F	do.	Es. coli; Enterococcus
8	52	M	hyperemic	Es. coli
9	40	M	pale, hypotrophic	Staphylococcus
10	70	M	do.	Es. coli; B. proteus
11	63	M	do.	Es. acidi lactici; Butyribacterium (fecaloid material)
12	44	M	do.	B. proteus; Staphylococcus
13	63	M	do.	Es. coli; Staphylococcus
14	43	M	do.	B. proteus
15	75	M	do.	B. aerogenes; paracolon bacillus
16	65	M	slight congestion	Es. acidi lactici
17	63	M	pale hypotrophic	Es. coli; B. proteus
18	62	M	do.	Enterococcus; Butyribacterium (fecaloid material)
19	37	M	do.	Es. coli
20	66	M	do.	B. proteus
21	46	M	do.	B. pyocyaneus
22	24	M	congested	Enterococcus; Es. coli
23	77	M	pale, hypotrophic	B. pyocyaneus; Es. coli; paracolon bacillus
24	41	M	hypotrophic	B. proteus; Es. coli
25	70	M	congested	B. pyocyaneus; Staphylococcus

*All specimens were negative for parasites and yeasts.

TABLE 11.—Observations on Material Obtained Through Miller-Abbott Tube*

Case	Age	Sex	Bilious Specimens	pH	Microscopic Findings	Parasites	Bacteria	Comment
1	29	F	I, 2 ft. II, 4 ft.		cholesterol and calcium carbonate crystals	neg.	neg.	
2	45	F						3 trials; intubation not done
3	48	F					do.	
4	67	F	I, 2 ft. II, 4 ft. III, 6 ft.	7.0 6.5 6.0	calcium carbonate crystals	do.	Staphylococcus; coliform bacillus	
5	31	F	I, 2 ft. II, 4 ft. III, 6 ft. IV, 8 ft.	7.5 6.5 6.0 5.5	calcium carbonate crystals	do.	respiratory flora (Strep.); Gaffkya tetragenus	
6	68	F	I, 2 ft. II, 4 ft. III, 6 ft.	7.5 6.5 5.5	calcium bilirubinate, phosphate, carbonate crystals	do.	neg.	

*All specimens were negative for yeasts.

TABLE 11.—*Continued*

Case	Age	Sex	Bilious Specimens	pH	Microscopic Findings	Parasites	Bacteria	Comment
7	33	F	I, 2 ft. II, 4 ft. III, 6 ft.	6.5 7.0 6.5	bile pigments and salts	Giardia	N. catarrhalis; Staphylococcus	
8	52	M						no trial
9	40	M	I, 2 ft. II, 4 ft. III, 6 ft.	4.5 4.5 5.5	carbonate crystals	neg.	neg.	
10	70	M	I, 2 ft. II, 4 ft. III, 6 ft.	7.5 6.5 6.5	bilirubinate and carbonate crystals	do.	do.	
11	63	M	I, 2 ft. II, 4 ft. III, 6 ft.	7.5 6.5 6.0	do.	do.	B. coli	
12	44	M	I, 2 ft. II, 4 ft. III, 6 ft.	7.5 7.0 6.5	do.	do.	respiratory flora (Strep.); N. catarrhalis	
13	63	M	I, 2 ft. II, 4 ft. III, 6 ft.		do.	do.	do.	
14	43	M	I, 2 ft. II, 4 ft.		do.	do.	neg.	
15	75	M	I, 3 ft. II, 6 ft.	7.5 7.5	do.	do.	Staphylococcus; Streptococcus	
16	65	M						trials neg.
17	63	M	I, 2 ft. II, 4 ft. III, 6 ft. IV, 8 ft.	7.5 7.0 6.5 6.0	do.	do.	B. pyocyaneus; Alkaligenes fecalis	
18	62	M						no trial
19	37	M	I, 3 ft. II, 5 ft. III, 8 ft.	7.0 7.0 6.0	carbonate crystals	Giardia (veg. form)	neg.	
20	66	M	I, 4 ft. II, 6 ft. III, 8 ft.	7.0 6.0 6.0	neg.	I, neg. II, Giardia (veg. form) III, Necator (ova)		
21	46	M	I, 3 ft. II, 5 ft.		do.	neg.	do.	
22	24	M	I, 2 ft. II, 4 ft. III, 6 ft.	7.0 6.5 6.0	bile pigments and salts	do.	Staphylococcus; B. proteus; coliform bacillus	
23	77	M	I, 2 ft. II, 4 ft. III, 6 ft.	7.5 6.0 7.5	do.	do.	B. proteus; Es. coli	
24	41	M	I, 2 ft. II, 4 ft. III, 6 ft.	7.5 6.5 6.0	carbonate and bilirubinate crystals	do.	Staphylococcus; N. catarrhalis; Streptococcus; B. coli	
25	70	M	I, 2 ft. II, 4 ft. III, 6 ft.	7.5 7.0 6.0	calcium carbonatecrystals; oleagenous material	do.	coliform bacillus	

ing, anaerobic bacillus called Butyribacterium, which corresponds to the bacterioid of Castellani and Chalmers. Growth of cysts, of which Monilia formed the bulk, was noted in the feces of 40 per cent of the cases. The bacteria and yeasts were essentially confined to the stools, whereas the parasites occurred both in the small intestine and in the feces. The percentage of incidence of these organisms in samples obtained from the three sites studied is shown in Table 12.

Also at Havana we have made intensive and repeated series of gastrointestinal studies of normal persons and patients with sprue.

TABLE 12.—Parasites, Yeasts and Bacteria

	Parasites, %	Yeasts, %	Bacteria,* %
Feces	56	40	72
Curettage	0	0	8
Miller-Abbott tube	12	0	0

*Butyribacterium.

Profound changes, although not pathognomonic of sprue, were found nevertheless in all patients with advanced cases. Examples are shown in Figures 23–28.

Before discussing the details of therapy in the next chapter, the profound changes induced in the bone marrow cells and perhaps in all other cells of the bodies of persons who have addisonian pernicious anemia, macrocytic anemia or macrocytic anemia of sprue warrant a brief résumé. In patients with proper diagnosis and adequate treatment the results are clearcut and dramatic. In uncomplicated cases there are restitution of the red blood cells, white blood cells and platelets and reduction in the size of the red blood cells. The rapid increase in number of reticulocytes in the peripheral blood, which in some instances has exceeded 60 per cent, is followed by an increase in the number of mature red blood cells, white blood cells and platelets and the hemoglobin content. Coincidentally the bone marrow becomes less and less hyperplastic, and if adequate treatment is continued long enough

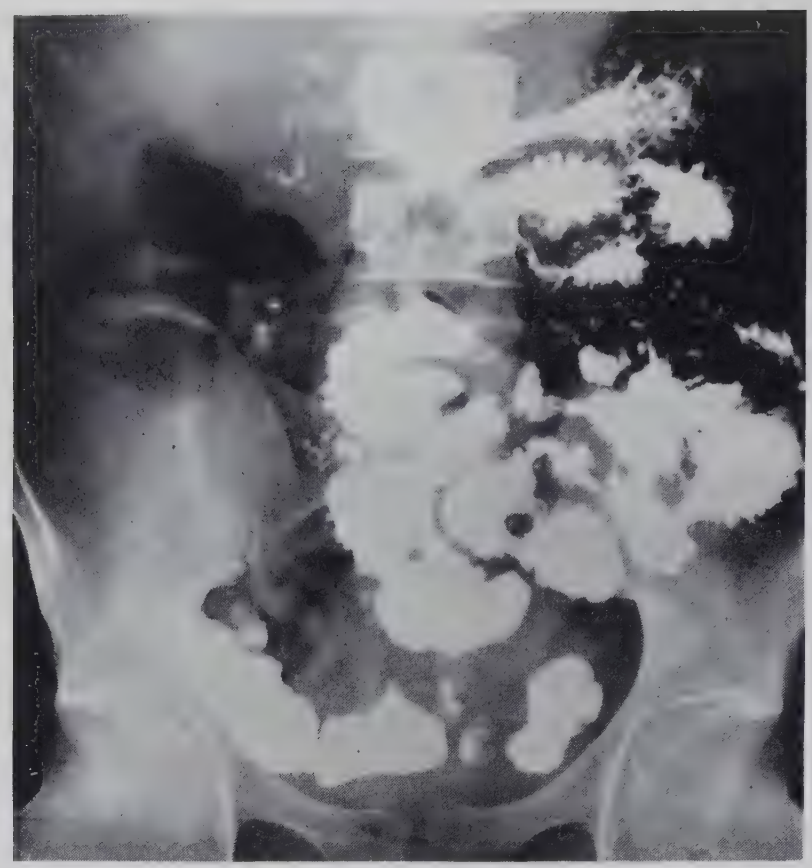


Fig. 23.—Case of tropical sprue. The barium meal is broken up in many areas; dilatation and spasm are evident. The patient received no specific treatment preceding base-line determinations.

Fig. 24.—Same case as preceding, several weeks later but before folic acid therapy. The barium column is broken up into isolated dilated segments, with striking distribution into numerous irregularities which warrant the description of "moulage" of barium.

Fig. 25.—Same case. Liquid, yellow stool passed the night before the gastrointestinal film, Figure 24, was made.

Fig. 26.—Same case, after one month of folic acid therapy. The barium column empties normally from the stomach into the small intestine and is no longer broken into isolated segments but is continuous. Alimentary tract spasm and dilatation have disappeared.

Fig. 27.—Same case. Stool passed just before the gastrointestinal series, Figure 26, was begun. The color was normal; contrast with Figure 25.

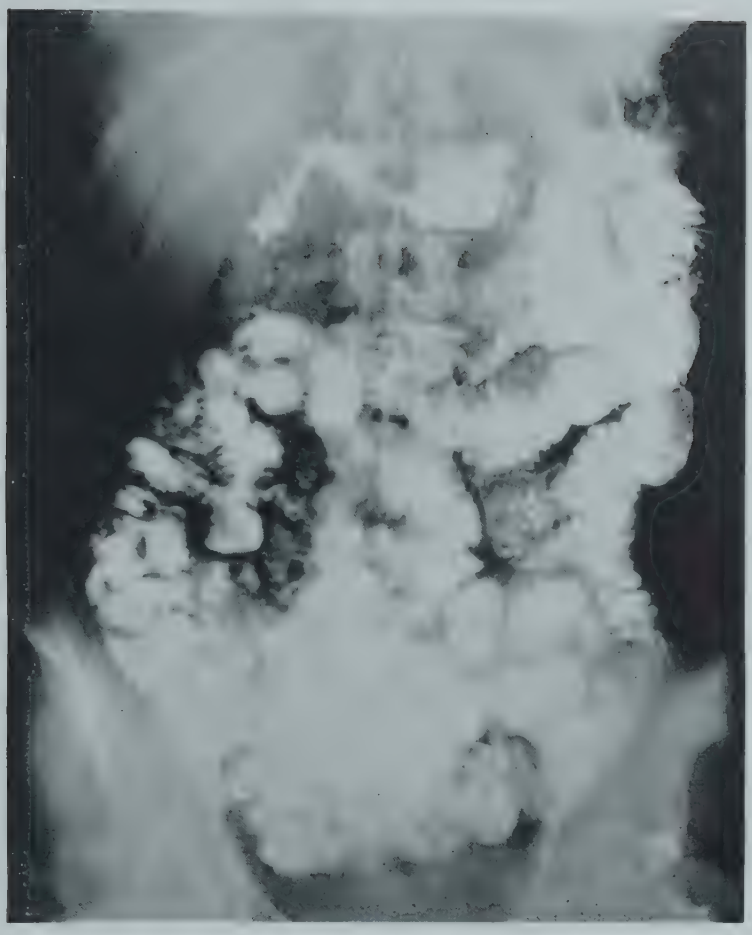


Fig. 28.—Film of normal person who has never had sprue, made with the same technic and on the same morning as that in Figure 26. The barium column is neither segmented nor interrupted but moves continuously. There is little or no significant difference between it and Figure 26.

the bone marrow and peripheral blood return to normal. This remarkable hemopoietic response is paralleled by an equally outstanding clinical response. About the fourth day of therapy there are a tremendous increase in appetite and a revival of interest in life. The burning and soreness of the tongue and other parts of the alimentary tract disappear; the papillae often re-form over the tongue; and the diarrhea, which so often is present in persons with sprue in relapse and frequently is present in persons with pernicious anemia and nutritional macrocytic anemia during relapse, tends to disappear and the stools tend to become normal. As remission progresses, the patient becomes stronger and stronger.

4. FOLIC ACID THERAPY

The discovery that folic acid is effective in the treatment of addisonian pernicious anemia, nutritional macrocytic anemia, sprue and the macrocytic anemias of pregnancy and pellagra marks the culmination of an epoch in the studies of these diseases. Its use in controlled experimentation is opening a new era in the understanding of the pathogenesis of some types of macrocytic anemia.

The basic clinical problem in treating macrocytic anemia is one of correct diagnosis. The objective in the treatment of every patient who has macrocytic anemia in relapse is the restitution of the red and white blood cell, platelets and hemoglobin values, the reduction of the red blood cells to normal size and the relief of all his symptoms so that he becomes completely rehabilitated. To realize this objective it is necessary to make a thorough study of the patient and of his blood. Once the diagnosis is made, general therapeutic measures that will promote physical rest and mental serenity should be instituted. A proper diet should be stressed throughout the period of convalescence and thereafter for the remainder of the patient's life. He should be treated by physiotherapy for any disturbances of gait and locomotion. Transfusion should be given, if necessary, to save life. Coexisting diseases should be treated and every effort made to eradicate them. During convalescence the physician must remind patients with

anemia to avoid unnecessary fatigue; many of them are old and their heart function is not what it once was. Dramatic recovery can be expected when folic acid is given promptly, efficiently and adequately to properly selected patients.

In the management of these cases I follow these five principles:

1. Make a positive diagnosis early in the course of the disease.
2. Institute persistent and intensive therapy promptly.
3. Prescribe an amount of folic acid in excess of that just necessary to maintain the patient's blood at his normal level.
4. Prescribe a diet rich in animal proteins.
5. Treat promptly all coexisting diseases and any complicating factors.

The response to treatment with folic acid parallels that afforded by potent liver extract. The speed of blood regeneration, however, is certainly no greater and may be slightly less than that which follows administration of the best of liver extracts. The blood level reaches the same height. I have seen patients respond to folic acid when it was given orally, subcutaneously, intravenously or intramuscularly. Dr. Carl Moore has found that patients can absorb enough folic acid even when it is given by enema to effect a response. He noted, however, that the response was greater when one-tenth the dose given by rectum was given by mouth. We have had many patients with macrocytic anemia associated with leukemia, aplasia of the bone marrow and other conditions who did not respond to folic acid whether it was administered by mouth or was injected. Not one of our patients with pernicious anemia, sprue or the macrocytic anemias of pregnancy or pellagra failed to respond to adequate dosage, irrespective of the route of administration. In no instance has a patient failed to respond to 20 mg. doses of folic acid by one route of administration and then responded when it was administered by a different route. In no instance has liver extract proved effective after 20 mg. doses of folic acid failed to produce a remission.

There is considerable variation in the dosage of folic acid required by different patients. I have observed some response in

a few patients when it was administered daily in doses of 3 mg. by mouth or 1 mg. parenterally. Three patients failed to respond to a daily oral dose of 5 mg. and later responded to 10 mg. Another patient who failed to respond to a daily parenteral dose of 5 mg. did respond when the dose was increased to 20 mg. Still another, who failed to respond to a daily dose of 2 mg. administered parenterally, subsequently responded when 10 mg. was given. Two patients who did not respond satisfactorily to 10 mg. by mouth responded well when the dose was raised to 20 mg. The minimal and optimal doses differ from person to person and even in the same person from time to time, so that the average dose probably has little meaning for the individual patient. Although we have not finally determined what dose to recommend, in view of satisfactory clinical experience I suggest a daily dose of 20 mg. given either by injection or by mouth. Large amounts of folic acid apparently can be given with impunity, for 400 mg. has been administered daily for three months without the patient's developing untoward symptoms.

We have made solutions of folic acid for injection by dissolving it in sterile saline made slightly alkaline with small amounts of soda bicarbonate. On occasion I have made 100 cc. of solution by dissolving 1 Gm. of sodium bicarbonate and 1 Gm. of synthetic L. casei factor in distilled water and autoclaving this mixture for 30 minutes under 15 lb. of pressure. Except in actual experimental work, we no longer weigh the folic acid on an analytical balance. It is now available in tablets which are sufficiently accurate for practical therapeutic purposes. The synthetic substance has standardized uniform potency. The harm done to the patient who gets ineffective and impotent preparations is incalculable.

Despite the fact that the blood levels improve following folic acid therapy, the patient's general nutritional status frequently warrants particular attention. The patient in severe relapse rarely is interested in food, and it is unlikely that he has been consuming

an adequate diet. As a rule, the appetite increases tremendously after remission begins, and it is not unusual for him to consume large amounts of food. At this time it is of utmost importance to instruct him regarding a proper diet. Experience has taught that there is a great variation in the needs of individual patients. The patient with sprue or pellagra is usually considerably underweight and has a deficiency of many nutrients. Accordingly, he may need a diet that not only is high in calories but is rich in all the essential nutrients. In contrast, some persons with pernicious anemia are obese, and the caloric intake must be restricted without impairing the diet with respect to other nutrients. Some patients may have renal insufficiency, diabetes or other diseases which require special dietary control. In such instances the diet should be prescribed for the individual patient and planned with great care. Too often patients develop outright deficiency states as a result of subsisting on a diet prescribed as a therapeutic measure for a specific disease but without regard for its adequacy for the nutritional needs of the patient. As can be seen from the case histories reported later in this chapter, we prescribe the diet according to the patient's individual needs.

In some of our patients the macrocytic anemia is accompanied by hypochromic microcytic (iron deficiency) anemia. Naturally folic acid will not replenish the iron supply. In such cases optimal doses of iron are given and diets rich in iron prescribed. A number of our patients have subsisted for many years on diets which supply far less than the optimal amounts of the essential nutrients. When a deficiency of one or more nutrients is found, we administer the needed substance or substances in addition to recommending a diet which furnishes all the nutrients in excess of normal requirements.

In the practice of medicine, absorption in the treatment of one disease too often precludes the detection of associated diseases. The patient must be treated not only for his anemia but for any associated diseases or conditions which would prevent or

retard his prompt return to good health. The presence of infections may retard blood regeneration despite ample therapy. Every effort should be made to find and to treat all likely foci of infection. In some instances surgery is indicated, and in these cases folic acid therapy should be continued.

In November, 1945, Dr. Carl F. Vilter, Dr. Richard Vilter, Virginia Hawkins and I initiated a study to test the efficacy of folic acid in maintaining persons with addisonian pernicious anemia. Each of the 24 patients selected for study had been maintained on liver extract under observation for a number of years. They were told that it was proposed to discontinue the liver extract and to give 30 mg. of folic acid three times a week. Each one volunteered to co-operate in the study. After complete clinical and laboratory examinations had been made the first dose was given.

After five to eight months on folic acid therapy, four of the 24 patients with pernicious anemia noted the gradual onset of numbness and tingling sensations in the hands and feet, unsteadiness of gait and moderate stiffness in the extremities. Neurologic signs appeared and gradually progressed until there was definite evidence of involvement of the posterior columns and the peripheral nerves in four cases, of the lateral columns in three, and of the cerebral cortex in one. None of these patients had had detectable neurologic involvement of any type when a diagnosis of pernicious anemia was first made or when folic acid therapy was initiated. The neurologic status of one patient who had signs of active peripheral neuritis at the beginning of the study remains unchanged after one year on folic acid therapy. No subjective or objective improvement occurred when the dose of folic acid was increased from 50 to 500 mg. daily, whereas the intramuscular injection of 5 cc. of liver extract daily was followed by very satisfactory convalescence in three of the patients and the fourth is showing more gradual improvement.

Our observations up to the present time suggest that in most

cases folic acid will maintain for at least a year the increased red blood cell and hemoglobin levels which have been produced either by folic acid or by liver extract. Although final conclusions cannot be drawn without further study, from our results so far it seems evident that folic acid does not always prevent the occurrence of combined system disease nor does it reverse the degenerative process once it has begun. These observations suggest that until more is known of the mode of action of folic acid in hemopoiesis and until a factor essential to the preservation of the central and peripheral nervous systems is found, liver extract is the safest and most effective therapeutic agent for the routine treatment of pernicious anemia.

Dr. Robert E. Stone and I have been studying a large series of persons with nutritional macrocytic anemia and another series with addisonian pernicious anemia. We have observed that combined system disease tends to develop in some patients with pernicious anemia after four to 12 months on folic acid therapy. No evidence of combined system disease has appeared in any patient with nutritional macrocytic anemia after 12 months of observation.

Since December, 1945, Dr. Ramon Suarez and Dr. Ramon Suarez, Jr., in Puerto Rico, and I have been attempting to determine whether folic acid administered by mouth can be substituted for liver extract administered parenterally in the treatment of patients who have chronic cases of sprue. Liver extract therapy in 28 patients who had been treated from one to 12 years was terminated. The following blood values were found at this time. But one of the 28 patients had a red blood cell count of 5,000,000 per cu. mm.; seven had over 4,000,000 red blood cells, and the remaining 20 had less than 4,000,000. All but four showed macrocytosis despite the fact that they had received parenteral injections of liver extract three times a week. In three of the patients with macrocytosis selected arbitrarily, the bone marrow showed 3.4, 3.4 and 9.6 per cent megaloblasts, respectively. These findings

and the clinical appraisals indicated that many of these patients with chronic sprue had not exceeded a certain level of response. Three weeks after the termination of liver extract therapy, baseline determinations were made, and a week later folic acid therapy was started. Each patient was given 10 mg. of folic acid twice a week by mouth. Within a week 19 of them volunteered that they noted some improvement. These 19 also showed a slight increase in reticulocytes and subsequently an increase in the red blood cell count. Nine showed no improvement after six weeks on folic acid therapy. One patient who had had troublesome diarrhea for many months improved so much and the stool became so hard that she did not wish further therapy. A few days after folic acid was discontinued, the diarrhea recurred and was not brought under control until 10 mg. of folic acid was once more administered daily. Our results up to the present time indicate that folic acid is a safe and satisfactory substitute for liver extract in the treatment of sprue.

Dr. L. Joe Berry and I have made some interesting observations on the effect of folic acid on the red blood cell count of supposedly "normal persons." Twenty-five persons — college students, faculty members and technicians — all apparently in excellent health, were selected for observation. Five had red blood cell counts below 4,000,000; the other 20 had red blood cell counts of 4,000,000 or more. To each of these persons 50 mg. of folic acid was administered by mouth every other day for two months. Each of the five persons whose initial red blood cell count had been less than 4,000,000 showed an increase to 4,400,000 or more, whereas the other 20 whose initial red blood cell count had been above 4,000,000 showed no response. It is interesting to note that nine of the subjects, including the five whose blood values were affected by folic acid, gave a history of subsisting on a diet which we considered inadequate in animal proteins.

Sensitivity reactions to the parenteral administration of liver

extract were reported in 17 per cent of the cases in a large series studied by Schwartz and Legere.[72] Death following the administration of liver extract was reported by Morgans.[73] As more and more parenteral injections are given, sensitivity to liver extract may become more common. Doan and his associates[61] and I[57] independently have observed that folic acid is a safe and satisfactory substitute for liver extract in producing blood regeneration and maintaining satisfactory blood levels in pernicious anemia. Since there is evidence that it does not protect against the neural disturbances which frequently develop, we do not suggest its use in the routine treatment of pernicious anemia until a factor essential to the preservation of the central and peripheral and nervous systems is found and administered with folic acid.

In persons with macrocytic anemia in relapse dried powdered brewers' yeast, yeast concentrate or liver extract may produce a response out of all proportion to the folic acid content of these preparations. Tests of both liver extracts and yeast concentrates for folic acid content have disclosed that some of them which were almost devoid of folic acid caused a hemopoietic response. These observations suggest that folic acid is not the major antianemic substance in these compounds. From a practical point of view, however, it must be stressed that it is a remarkably effective hemopoietic substance, whether administered by mouth or by injection.

To most physicians it is evident that the evaluation of the efficacy of liver, liver extracts, ventriculin, yeast and folic acid as therapeutic agents in the macrocytic anemias in which they are effective is difficult. It will seldom be possible to test satisfactorily the comparative value of these substances because such tests must be made under rigidly controlled conditions in order to yield information of practical value. Few physicians are in a position to carry out such tests. The choice of the therapeutic agent necessarily will be determined to some extent by the geographic location of the patient and the availability of the preparation. Many patients will try various therapeutic substances and finally decide

which one they prefer. To some patients the administration of any therapeutic agent by injection presents a major difficulty, whereas to others it is a minor problem. Folic acid has an advantage over some of the other antianemic substances in that it is effective administered orally or by injection. The parenteral route is more satisfactory in some cases because by this means therapy can be kept under the absolute control of the physician.

I know of no better way to illustrate the effectiveness of folic acid therapy than to describe rather briefly the course in three representative cases.

CASE 1.—Addisonian pernicious anemia. J. D., a man aged 68, was admitted to Hillman Hospital in October, 1945, with complaints of weakness, dizziness, pains in the legs and soreness of the tongue.

Family history.—Irrelevant.

Past history.—The patient was well until 10 years before, when he was found to have hypertension.

Present illness.—Three years before this admission he began having occasional "weak, dizzy spells" and had become progressively weaker despite the fact that his appetite remained good. He continued to eat a liberal well balanced diet and to work regularly as a bricklayer. Eighteen months before the present admission he came to the Nutrition Clinic and a diagnosis of addisonian pernicious anemia was made. On this occasion and again nine months later he was hospitalized. He had a maximal hemopoietic response following treatment with reticulogen and was discharged from the hospital feeling perfectly well. He went back to work and refused to return for follow-up studies. After his second discharge from the hospital, he worked regularly for six months as a bricklayer. He stopped working two months before the present admission because he felt tired. His appetite decreased and there developed a burning sensation of the tongue which was aggravated when he ate hot or highly seasoned foods. A month before this admission he began having numbness, tingling and cramping of his hands and feet and dizziness. The dizziness became so severe that he was afraid to go out alone and for this reason he decided to come to the hospital.

Physical examination revealed a well developed, somewhat overweight, elderly man. The conjunctivas, tongue, palms and nail beds

were extremely pale. The heart was enlarged. The lungs and abdomen were normal. Blood pressure was 115/65. The remainder of the physical examination was negative.

Neurologic examination revealed normal co-ordination, locomotion, sensitivity, equilibrium and reflexes. The calf muscles were sensitive to deep pressure.

Laboratory findings.—Gastric analysis showed no free hydrochloric acid, pepsinogen or rennin after histamine stimulation. The red blood cell count was 2,000,000, the hemoglobin content 8 Gm. (52 per cent), and reticulocytes 0.3 per cent. The bone marrow was hyperplastic and showed typical megaloblastic arrest.

Course.—After the base-line studies were completed he was given 10 mg. of synthetic folic acid daily by mouth. The third day after the initiation of therapy he stated that his tongue had stopped burning, that he felt his strength "surging back" and that he had "a taste for food." His appetite increased tremendously. The reticulocyte values began to rise on the third day of therapy and reached a peak of 18 per cent on the fifth day. By the time he was discharged after 43 days of therapy, the red blood cell count had risen to 5,000,000 and the hemoglobin content to 14.8 Gm. (96 per cent) (see Fig. 6, p. 52). Since he was overweight, he was instructed regarding the 1,800 calorie diet.

Suggested 1,800 Calorie Diet

BREAKFAST	DINNER	SUPPER
Fruit, 1 serving	Lean meat or fish, 4 oz. (¼ lb.)	Lean meat or fish, 4 oz. (¼ lb.)
Eggs, 2	Vegetable, 2 servings	Vegetable, 2 servings
Toast, 1 slice	Bread, 1 slice	Bread, 1 slice
Butter, 1 teaspoon	Butter, 1 teaspoon	Butter, 1 teaspoon
Milk, 1 cup	Fruit, 1 serving	Fruit, 1 serving
Coffee, if desired	Milk, 1 glass	Milk, 1 glass
	Coffee or tea, if desired	Coffee or tea, if desired

NOTE.—No sugar should be used on food or in cooking. No fats other than the 3 teaspoons of butter or oleomargarine are to be used on food or in cooking food. This diet supplies approximately 100 Gm. of protein.

Two days after he was discharged he returned to work. He again refused to take therapy at home and four months later the blood values were falling.

Case 2.—Nutritional macrocytic anemia. C. W., a man aged 72, was admitted to Hillman Hospital in November, 1945, with complaints of dizziness, diarrhea, weakness and loss of 30 lb. in six months.

Family and past histories.—Irrelevant.

Present illness.—All his life he had lived on a farm, had worked hard and had considered his health to be perfect until six months before admission. His appetite had always been excellent, but although a liberal and varied food supply had always been available he disliked meat, poultry and fish and rarely ate them. He drank very little milk and seldom ate more than one egg a day. Two years before admission he retired and spent much of his time working in his garden. Eighteen months later he noticed dizziness when he stooped over. A few weeks later there developed diarrhea characterized by the passing of six to eight semisolid, brown stools daily. About this time his appetite failed and he lost weight rapidly. Within a week he became too weak to work in his garden and was forced to spend the greater part of the day in bed. He observed that when he tried to stand up his feet were "asleep" and he was unable to walk. His mouth and tongue were so sore that eating was painful. He restricted himself to a diet of cooked cereals, milk, soup and occasionally an egg. He grew progressively weaker and began to have "fainting spells" which so alarmed his family that they brought him to the hospital.

Physical examination revealed a well developed, undernourished, weak man. The conjunctivas, skin and nail beds were extremely pale. The tongue was swollen, atrophic and reddened along the margins and at the tip. The heart, lungs and abdomen were normal. Blood pressure was 117/75.

Neurologic examination revealed tenderness of the calf muscles on deep pressure. Vibratory perception was decreased in the hands and feet. Position sense of the toes was intact. There was mild pitting edema of the ankles.

Laboratory findings.—The Kahn reaction was negative. The stools were soft to liquid in consistency, were light brown and contained no blood or pathogenic bacteria; the fat content was normal. Fluoroscopic examination of the gastrointestinal tract revealed no abnormalities. The gastric juice contained 52 degrees of free hydrochloric acid, pepsinogen and rennin after histamine stimulation. The red blood cell count was 1,800,000, hemoglobin content 9 Gm. (59 per cent) and reticulocytes 0.4 per cent. The bone marrow was hyperplastic and showed arrest at the megaloblastic level.

Course.—On the basis of clinical and laboratory findings, a pro-

visional diagnosis of nutritional macrocytic anemia was made. Many additional gastric studies showed similar results, and after satisfactory base-line determinations had been made he was given 10 mg. of synthetic folic acid daily by mouth. On the second day of treatment he volunteered the information that his tongue no longer burned or felt sore. On the third day he ate all the food offered and asked for additional servings, whereas during the preliminary period of observation he had refused to eat anything but a little cereal or a bowl of soup. On the fourth day the reticulocyte values began to rise and reached a peak of 21 per cent on the seventh day. After 21 days of folic acid therapy the red blood cell count had risen to 4,000,000 and the hemoglobin content to 14.8 Gm. (96 per cent) (see Fig. 7, p. 53). By this time he had gained 10 lb. and insisted on taking a long walk daily. He was instructed to eat the following high calorie diet.

Suggested 3,000–3,500 Calorie Diet

Breakfast

Fruit juice, 1 glass
Cereal, large serving (¾ cup)
Eggs, 2
Bacon, if desired
Toast, 2 slices
Butter, 1 tablespoon
Cream, ½ cup (for cereal and coffee)
Milk, 1 glass
Coffee, if desired

10:00 A. M.: Eggnog, 1 cup (see recipe below)

Dinner

Lean meat, chicken or fish, 3 oz.
Potato, macaroni, spaghetti, noodles or dried beans or peas, 1 serving (½ cup)
Vegetable, 1 serving of green or yellow vegetable (May be cooked or used as a salad. If cooked, add 1 tablespoon of butter or other fat. If used as a salad, add 1 tablespoon of mayonnaise or oil.)
Bread, 2 slices
Butter, 1 or 2 tablespoons
Dessert, 1 serving (½ cup)
Milk, 1 glass

2:00 P. M.: Eggnog, 1 cup

4:00 P. M.: Eggnog, 1 cup

Supper

Lean meat, chicken or fish, 3 oz.
Potato, macaroni, spaghetti, noodles or dried beans or peas, 1 serving (½ cup)
Vegetable, 1 serving of green or yellow vegetable (May be cooked or used as a salad. If cooked, add 1 tablespoon of butter or oleomargarine. If used as a salad, add 1 tablespoon of mayonnaise or oil.)
Bread, 2 slices
Butter, 1 or 2 tablespoons
Dessert, 1 serving (½ cup)
Milk, 1 cup

8:00 P. M.: Eggnog, 1 cup

RECIPE FOR EGGNOG

3 eggs
3 cups milk
2 tablespoons sugar
Vanilla, chocolate, or any flavoring desired.

Beat eggs well. Add sugar. Add milk and flavoring. Beat well. Makes 4 cups.

NOTE.—This diet supplies approximately 150 Gm. of protein. The patient was discharged from the hospital at this time and the following day began working in his garden and aiding his wife with the housework.

CASE 3.—Tropical sprue. C. C., a Cuban, aged 54, was admitted to the Calixto Garcia Hospital, Havana, Cuba, in January, 1946, with complaints of diarrhea of three months' duration and weakness which had progressed steadily since the onset of the diarrhea.

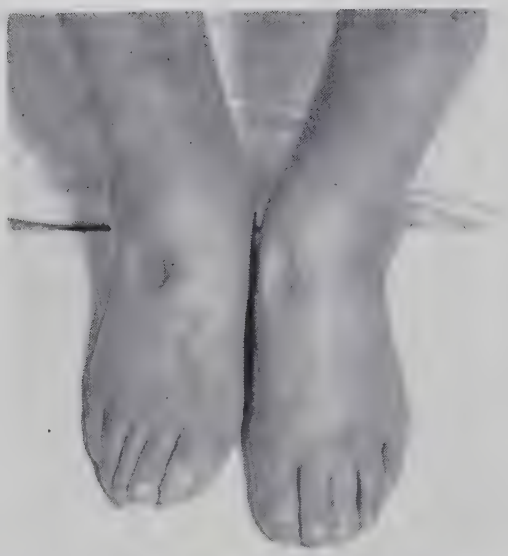
Family history.—Irrelevant.

Past history.—Since he was 20 years old, he had worked steadily as a carpenter.

Present illness.—His health always had been good and his appetite excellent until the present illness. Although he ate a liberal amount of food, his diet included a very small amount of animal protein foods and large amounts of rice, corn, flour, dried beans and pork fat. Three months before admission he began having from six to seven large, light-colored, foul-smelling liquid stools a day. The bowel movements were accompanied by much gas and a burning sensation of the rectum. Soon after onset of the diarrhea his mouth and tongue became sore and he felt extremely weak. His appetite remained fairly good for the first 15 days of his illness. Then severe anorexia developed and soon he became too weak to work. By this time his mouth and tongue were so sore that he was unable to eat anything but bread soaked in coffee and soup. Three days before admission he noticed severe swelling of the ankles and feet. This so alarmed him that he came to the hospital seeking treatment.

Physical examination revealed an emaciated, pale, extremely weak man (see Plate IV, fig. 1). His weight showed a loss of 35 lb. since onset of the illness, but since he had considerable edema of the ankles the actual weight loss probably exceeded this amount. His hair was dry and brittle. His skin, which was loose and dry, had a profound icteric tint. The conjunctival scleras were yellow. His tongue was pale and the papillae were atrophied at the tip and along the edges. The abdomen was distended and tympanitic, and there was slight pain on deep palpation in the right quadrant.

Neurologic examination revealed slight exaggeration of the patellar reflexes and pain of the leg muscles on deep pressure. There was profound pitting edema of the ankles and feet (Fig. 29).



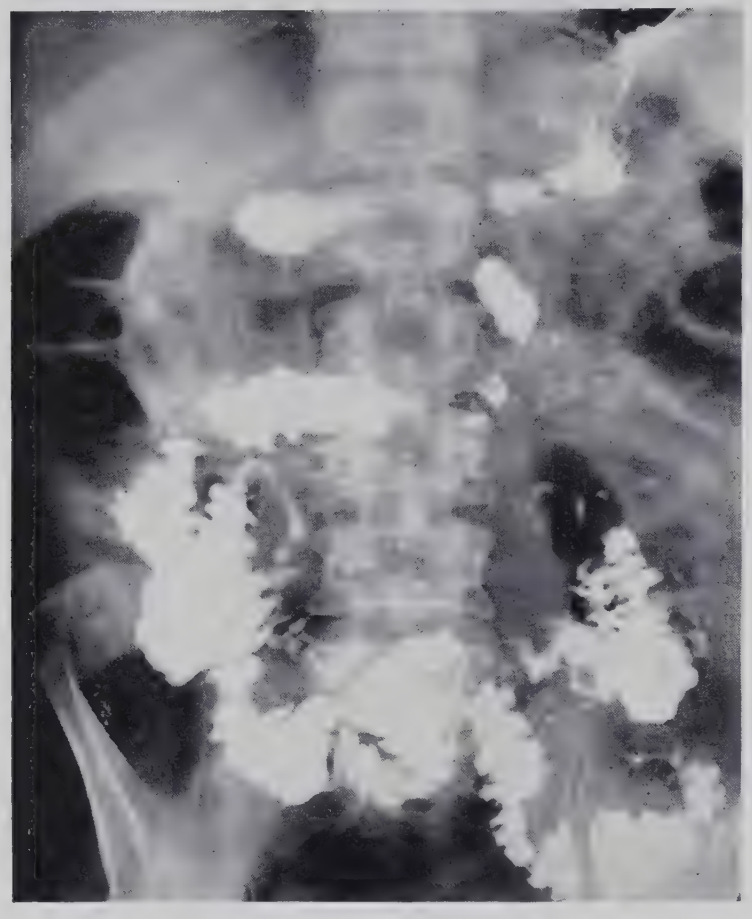


Fig. 29.—Patient C. C., with tropical sprue, showing pitting edema of ankles and feet.

Fig. 30.—Patient C. C. Film made during gastrointestinal studies before folic acid therapy. Forty-five minutes after barium had been given it had passed out of the stomach and been broken into a number of isolated areas. Dilatation and spasm are evident. The "stack of coins" effect is apparent.

Laboratory findings.—Base-line laboratory determinations made during the six days prior to the initiation of therapy gave the following results.

Chemical analysis of fat in the stools revealed: total fat, 1.56 per cent; neutral fat, 1.46 per cent; fatty acids, 0.11 per cent. The stools were yellow, liquid and smooth and contained undigested food particles. Analysis showed: reaction, pH 4.5; fatty acid, 15 cc. of 0.1 N sodium hydroxide per 10 Gm. of wet feces. Microscopic study disclosed no ova, cysts or parasites. Tests for occult blood gave negative results.

Stool culture gave essentially normal results, showing the presence of Escherichia coli, Enterococcus and Bacillus proteus. Culture of rectal scrapings obtained during proctoscopic examination gave essentially normal results, showing the presence of Bacillus proteus and staphylococcus. Culture of jejunal contents obtained by the Miller-Abbott tube gave essentially normal results, showing the presence of Neisseria catarrhalis, Staphylococcus, Streptococcus and Pneumococcus.

Proctoscopic examination revealed pale, hypertrophic and shiny mucosa.

Gastrointestinal roentgen studies showed that the barium column was broken into many isolated areas (Fig. 30). Gastric analysis revealed hypochlorhydria, with 25 degrees of free hydrochloric acid obtained in a 45 minute sample after histamine stimulation.

A glucose tolerance test after 40 Gm. of glucose was administered orally showed the following values: fasting, 90.9 mg. per cent; 30 minutes, 95.2 mg.; 60 minutes, 95.2 mg., and 90 minutes, 86.9 mg. per cent.

Blood examination revealed: red cells, 1,800,000; hemoglobin content, 3.8 Gm. (24 per cent); white cells, 6,950; reticulocytes, 2.1 per cent; serum proteins — total 5.1 Gm., albumin 3 Gm., globulin 2.1 Gm.

Bone marrow study on admission was made from sternal marrow obtained by means of the Turkel trephine. Two hundred white blood cells were counted, with the following differential values:

Cells	No.	%
Polymorphonuclears	120	60
Metamyelocytes	56	28
C myelocytes	2	1
B myelocytes	0	0
A myelocytes	0	0

Cells	No.	%
Basophils	2	1
Basophilic myelocytes	0	0
Eosinophils	4	2
Eosinophilic myelocytes	10	5
Plasma cells	2	1
Megakaryocytes	0	0
Primitive cells	4	2
Total	200	100
Number of nucleated R.B.C. per 100 W.B.C.		
Megaloblasts	12	
Early erythroblasts	6	
Late erythroblasts	9	
Normoblasts	15	
Total	42	

Course.—After the base-line studies were completed, the patient was given 10 mg. of folic acid daily by mouth. Two days after this therapy was initiated he was sitting up in bed smiling and asking for breakfast, whereas previously he had seldom opened his eyes, rarely smiled and never took anything but coffee for breakfast. There was an abrupt change in the character of the stools on the second day of therapy (see Plate IV, figs. 3 and 4). The reticulocyte value rose to 5.8 per cent and on the eighth day reached a peak of 33 per cent. By this time his food intake, which had not exceeded 400 calories during the six days he was in the hospital before therapy was initiated, increased to between 4,000 and 5,000 calories a day. After treatment for two weeks the bowel movements decreased to one a day and he no longer complained of burning of the rectum on defecation. By this stage of treatment the scleras and skin were normal in color and the edema of ankles and feet had disappeared (Fig. 31). He asked to be allowed to get up and help the orderly with the other patients on the ward and was allowed to do so. While he was in the hospital he gained 30 lb. and showed a striking change in appearance (Plate IV, fig. 2). The red blood cell count and hemoglobin content increased steadily, and by the time he was discharged from the hospital after 76 days on folic acid therapy they had risen to 4,620,000 and 15.2 Gm. (99 per cent), respectively.

A gastrointestinal series taken after 18 days of folic acid therapy (Fig. 32) showed a condition comparable to that in a normal person (Fig. 28, p. 75).

PLATE IV

Fig. 1 (*above left*).—Tropical sprue. Emaciated man before therapy.

Fig. 2 (*above right*).—Same patient two months later, after folic acid therapy. Note appearance of increase of weight.

Fig. 3 (*below left*).—Same case. Bowel movement in a 1 gal. jar just before therapy was started.

Fig. 4 (*below right*).—Same case, two days later. Abrupt transformation in character of the stool occurred after two days of folic acid therapy. Note the difference in color and texture between the upper and lower parts of the stool.

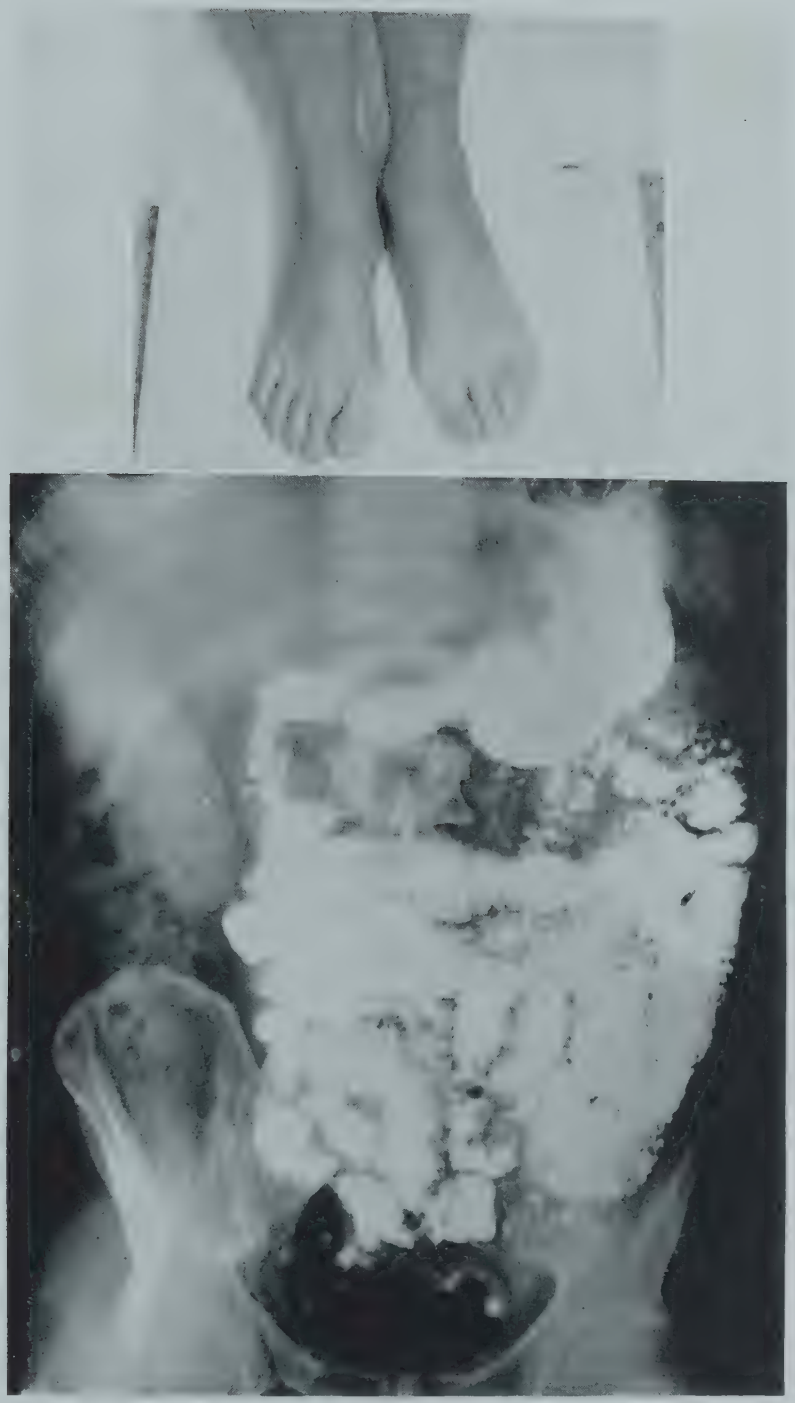


Fig. 31.—Patient C. C., showing ankles and feet two weeks after initiation of folic acid therapy. Many times the edema is not relieved by folic acid. Its exact mechanism is not known.

Fig. 32.—Patient C. C. Film made during gastrointestinal studies after 18 days of folic acid therapy. The barium column is continuous and there is no evidence of spasm or abnormal dilatation. Contrast this with Figure 30, before treatment, and compare with Figure 28.

A bone marrow study made on the fifty-first day of treatment showed the following values:

Cells	No.	%
Polymorphonuclears	86	43.0
Metamyelocytes	50	25.0
C myelocytes	3	1.5
B myelocytes	4	2.0
A myelocytes	5	2.5
Basophils	1	0.5
Basophilic myelocytes	1	0.5
Eosinophils	9	4.5
Eosinophilic myelocytes	35	17.5
Plasma cells	2	1.0
Megakaryocytes	1	.5
Primitive cells	3	1.5
Total	200	100.0
No. of nucleated R.B.C. per 100 W.B.C.		
Megaloblasts	6	
Early erythroblasts	11	
Late erythroblasts	20	
Normoblasts	109	
Total	146	

The impression was: a reactive normoblastic marrow which shows a good response to therapy, with still some evidence of megaloblastic arrest.

Another bone marrow study made on the seventy-fourth day of treatment showed:

Cells	No.	%
Polymorphonuclears	94	47.0
Metamyelocytes	54	27.0
C myelocytes	8	4.0
B myelocytes	5	2.5
A myelocytes	2	1.0
Basophilic myelocytes	1	.5
Eosinophils	6	3.0
Eosinophilic myelocytes	17	8.5
Plasma	1	.5
Megakaryocytes	1	.5
Primitive	6	3.0
Lymphocytes	5	2.5
Total	200	100.0
No. of nucleated R.B.C. per 200 W.B.C.		
Megaloblasts	0	
Early erythroblasts	3	
Late erythroblasts	24	
Normoblasts	37	
Total	64	

The impression was: essentially normal marrow except for an increased number of eosinophilic elements, showing a definite change toward normal since the fifty-first day of treatment.

The patient was discharged from the hospital after 76 days of folic acid therapy and two days later was back at his work as a carpenter. He has received no specific therapy since then but has come to the hospital once a month for follow-up studies. Studies made three months after his discharge from the hospital showed: red blood cells 5,190,000; hemoglobin, 15.8 Gm. (102 per cent); white blood cells, 9,100, and reticulocytes, 0.2 per cent (Fig. 33).

The patient was instructed to follow the diet shown below.

SUGGESTED 2,500–3,000 CALORIE DIET

BREAKFAST
- Fruit, 1 serving
- Eggs, 2
- Bread, 2 slices
- Butter, oleomargarine or other fat, 1 tablespoon
- Milk, 1 glass
- Coffee, if desired

DINNER
- Lean meat, poultry or fish, 1 serving (¼ lb.)
- Potato, root vegetable or rice, 1 serving
- Other vegetable, 1 or 2 servings
- Bread, 1 slice
- Butter, 1 level teaspoon
- Fruit, 1 serving
- Milk, 1 glass
- Coffee or tea, if desired

SUPPER
- Lean meat, poultry or fish, 1 serving (¼ lb.)
- Potato, root vegetable or rice, 1 serving
- Other vegetable, 1 or 2 servings
- Bread, 1 slice
- Butter or oleomargarine, 1 level teaspoon
- Fruit, 1 serving
- Milk, 1 glass
- Coffee or tea, if desired

BEFORE BED
- Milk, 1 glass

NOTE.—This diet supplies approximately 100 Gm. of protein.

He followed these instructions and gained an additional 12 lb. He has been free from diarrhea, has not missed a day's work and says that he feels "like a young boy."

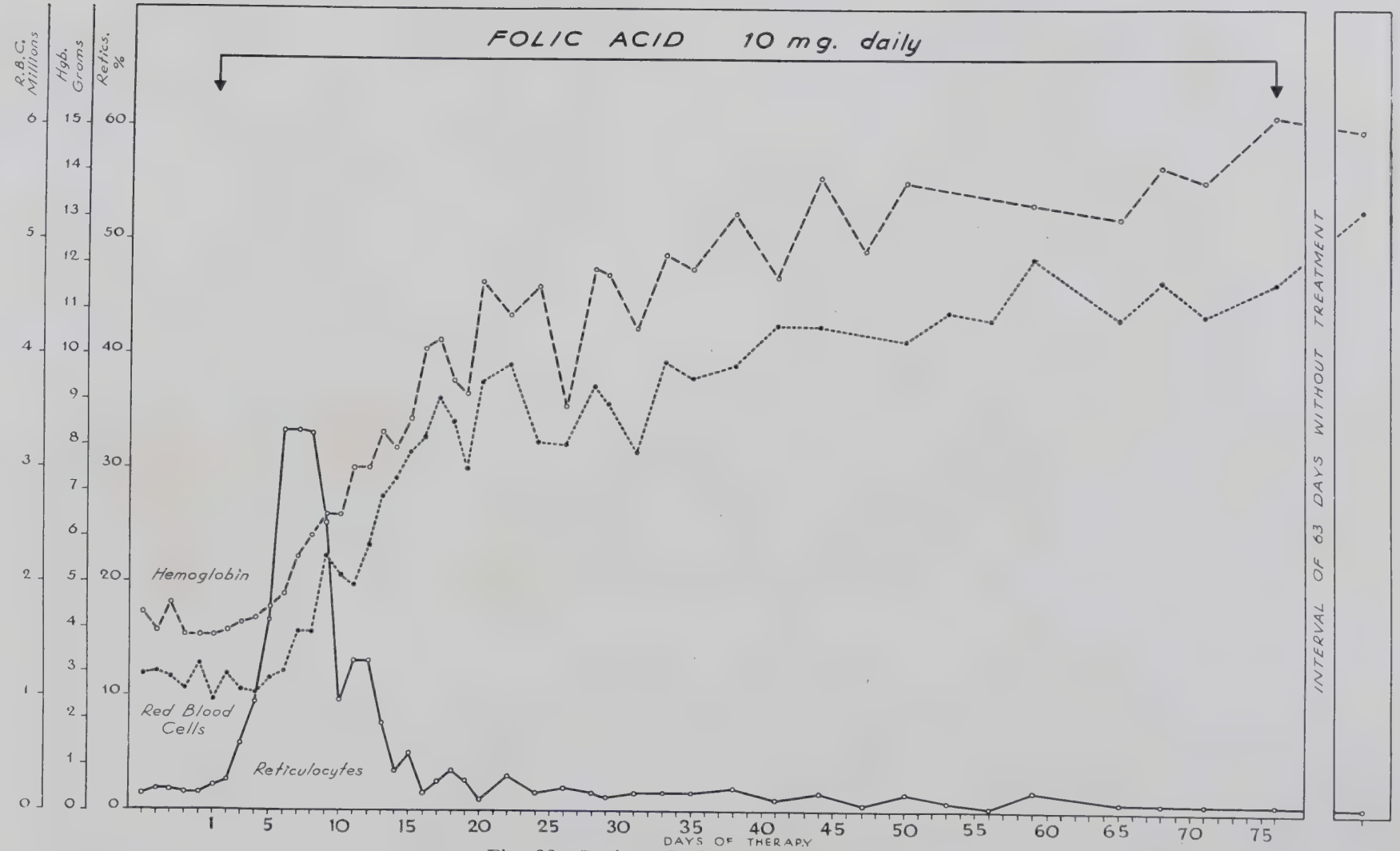


Fig. 33.—Patient C. C. Response to folic acid.

Wintrobe,[74] Castle and Minot,[75] Kracke[76] and Murphy [77] have described the response to liver and liver preparations so beautifully that to repeat a description here seems unwarranted.

Because folic acid has been used for such a comparatively short time, it is impossible to predict what increase in the life span of persons with addisonian pernicious anemia, nutritional macrocytic anemia or the macrocytic anemia of sprue can be brought about by its use. Already we have seen it restore their health and their ability to work. The outlook for patients who are sensitive to liver is more favorable than it ever has been.

It must be emphasized that neither my associates and I nor any of the other investigators have brought forth satisfactory evidence to warrant the assumption that folic acid prevents the neural disturbances which sometimes are associated with pernicious anemia. Insufficient folic acid therapy and the recurrence of complications may result in failure of the blood values to reach normal or to remain at normal levels. Inadequate treatment may come about because of the patient's unwillingness or inability to co-operate or because of the physician's failure to diagnose the disease properly or to treat the patient adequately. Fortunately the number of physicians who fail to do this is becoming smaller each year. I again wish to stress that there is no place for careless diagnosis and casual treatment when either liver, liver extract, ventriculin or folic acid is employed as a therapeutic agent. And failure of the physician to detect sprue, pernicious and other related anemias almost inevitably leads to a chain of unfortunate circumstances for the patient.

5. RECAPITULATION

Folic acid is the newest member of the vitamin family. "Folic acid" is the name originally given a substance obtained in a nearly pure form from spinach by Mitchell, Snell and Williams.[8] This substance supports a growth for two organisms frequently used in microbiologic investigations: Lactobacillus casei and Streptococcus lactis R (now termed Streptococcus faecalis). Its potency for S. lactis R was used as a guide in purification procedures, and "folic acid" is defined specifically as a growth factor for that organism. Three other rather similar crystalline compounds have been isolated from liver, yeast or other sources which, strictly speaking, cannot accurately be termed "folic acid." The synthetic material used in the studies reported here, unless otherwise stated, is identical with crystalline preparations isolated from liver independently by Stokstad and Pfiffner and associates. These and other substances that are somewhat similar occur in small amounts in liver, yeast and other foods. About 1/10 oz. of folic acid is contained in 1 ton of fresh liver. The Lactobacillus casei factor, pteroylglutamic acid, differs from the substance isolated from fermentation residue in that the latter contains two more molecules of glutamic acid. As far as I know, the fermentation factor, pteroyltriglutamic acid, has been found only in the fermentation product of corynebacterium. The pteroylglutamic acid conjugates in yeast and liver have a longer side chain. The vitamin B_c conjugate has six glutamic acid

residues. The folic acid conjugates in natural foods apparently correspond closely to the glutamic acid compound rather than to the so-called fermentation compound.

I have been able to show that either the Lactobacillus casei factor of liver or the fermentation Lactobacillus casei factor will stimulate the bone marrow of persons who are in the relapse stage of addisonian pernicious anemia, nutritional macrocytic anemia or the macrocytic anemia of sprue, of pregnancy and of pellagra.

Observations on the effect of the synthetic liver Lactobacillus casei factor (synthetic folic acid) have been confirmed and extended by Moore, Doan, Darby and Jones, my associates, me and others. My observations on the effectiveness of the fermentation Lactobacillus casei factor have not as yet been confirmed. The indications are that folic acid is as effective in producing blood regeneration and maintaining the blood at satisfactory levels in persons with addisonian pernicious anemia, nutritional macrocytic anemia and the macrocytic anemia of sprue as is liver extract but that it does not protect against the central and peripheral nervous system changes which frequently develop in persons with addisonian pernicious anemia nor will it reverse these neural changes once they have developed.

The finding of the specific effect of this agent on the cells of the bone marrow and perhaps on other cells opens a fresh and fertile field for the clinical investigator, who must now redefine the macrocytic anemias in the light of all the various loose threads which enter into the meshwork of their pathogenesis.

The effectiveness of pteroylglutamic acid, i.e., folic acid or the Lactobacillus casei factor, in the treatment of certain macrocytic anemias has been so definitely demonstrated that it must be accepted. I include pernicious anemia in the group of macrocytic anemias that respond to folic acid, yet I realize that its natural pathogenesis is somewhat different from that of the macrocytic anemias of sprue, pregnancy and pellagra and of nutritional macrocytic anemia. My working hypothesis is that folic acid is a

part of an enzyme system. We know that folic acid is widely distributed in foods. The greatest proportion of it occurs in the form of conjugates of higher molecular weight. For example, the conjugate of yeast (heptaglutaminate) contains six additional molecules of glutamic acid. Every observation made during the past 12 months has tended to show that persons with pernicious

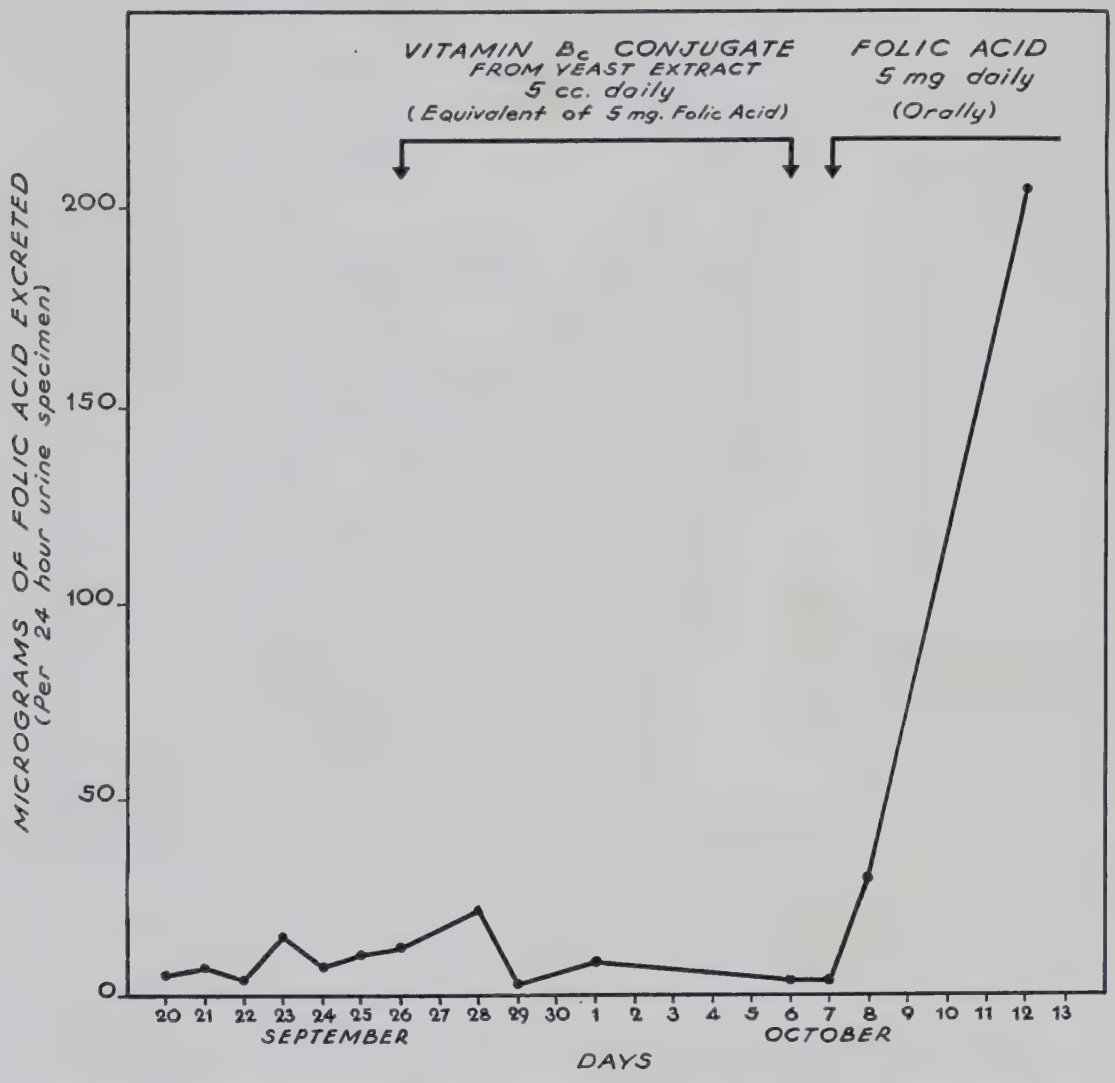


Fig. 34.—Urinary excretion of folic acid by a patient with addisonian pernicious anemia.

anemia are unable to liberate efficiently the folic acid from its conjugates. Many mammalian tissues contain enzymes, conjugases, that can convert these conjugates to folic acid. When we inject the conjugates into normal persons, the urinary excretion

of folic acid is increased. Patients with nutritional macrocytic anemia and with pellagra and one person with sprue have appeared to be somewhat more efficient in utilizing these naturally occurring conjugates than are patients with pernicious anemia, that is, if we consider the conversion of the conjugate to free folic acid as an index for measurement.

Bethell and Heinle and their associates and Spies, Stone, Scholz and Williams have all found that patients, including those with pernicious anemia, excrete a great deal of the pteroylglutamic acid administered to them (see Fig. 34). However, the ability to convert the vitamin conjugate to the free form efficiently seems to be reduced or greatly lagging in persons with addisonian pernicious anemia. To my way of thinking, the phenomenon behind this fact is a fundamental tissue disturbance affecting the biochemical system. If one gives sufficient dosage of the conjugate to a person with addisonian pernicious anemia in relapse, he will show a hemopoietic and clinical response. No evidence of excretion of the conjugate has been obtained, however, using microbiologic assay.

No satisfactory explanation has yet been given for the fact that large amounts of folic acid are required to produce a satisfactory hemopoietic response. When one considers that relatively little of a highly potent liver extract is needed, this naturally leads to the thought that perhaps the folic acid conjugates are stored in the body until a substance contained in liver extract liberates from them a material which acts on the cellular constituents of the bone marrow. It is a striking fact that relatively few milligrams of these various chemical substances are capable of profoundly affecting the bone marrow cells, and this in itself marks the culmination of an epoch in hematologic investigation.

It is doubtful that folic acid and the other chemical substances that will be found to act similarly will prove to be of importance in the treatment of leukemia, aplastic anemia, hemolytic anemia and drug idiosyncrasies associated with a leukopenic state.

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INDEX

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١٣: ٤٠ ١١ ٣٤